Prevention and treatment of scars

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Prevention and treatment of scars

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Contents

Chapter 1

General introduction

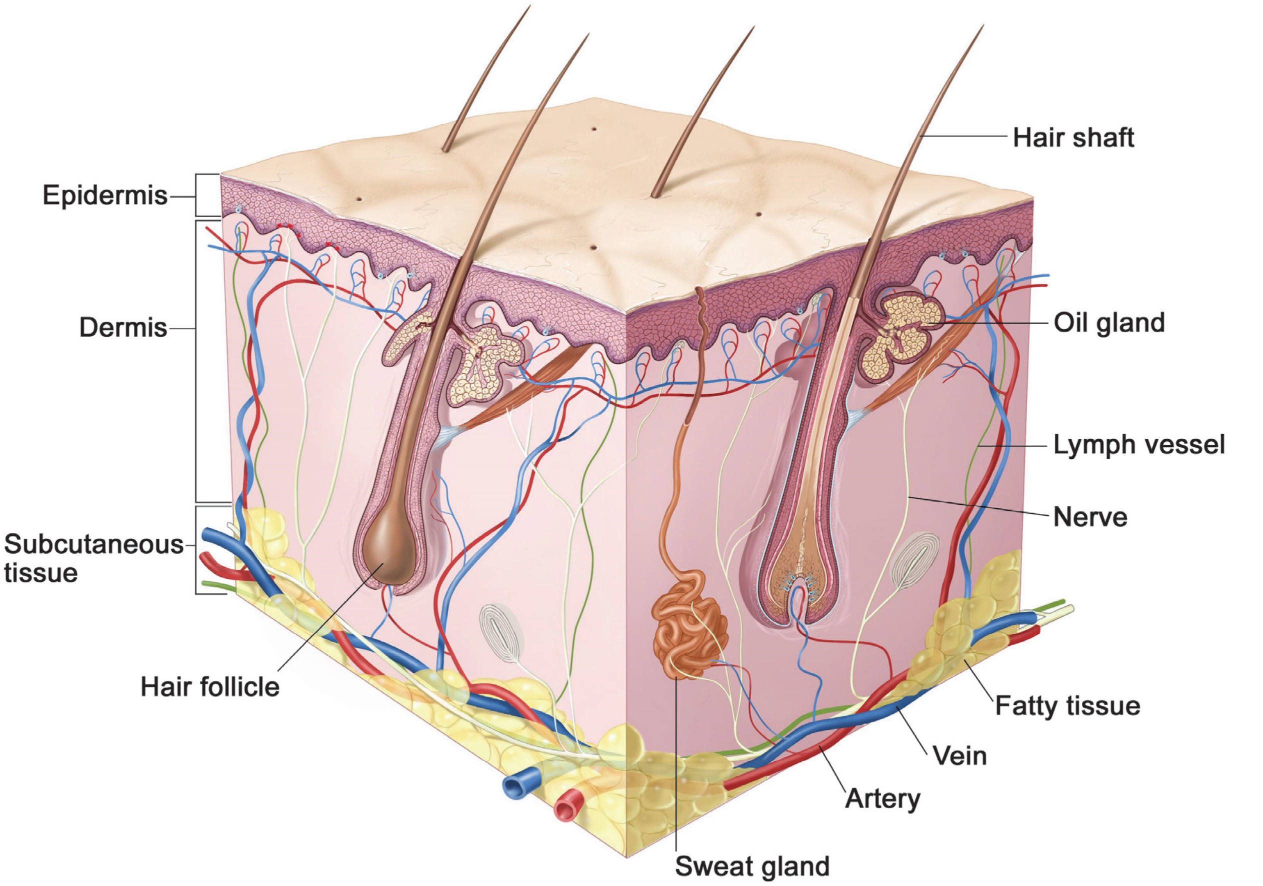


Figure 1. The skin consists of a top layer or epidermis and a second layer, the dermis. Subcutaneous fat is positioned underneath.

The skin is the largest organ in humans and plays a vital role in protecting the inner body. Besides its protective function, it serves a variety of other functions such as sensation, heat regulation, absorption, excretion and of course its aesthetic function. Unfortunately, our skin is also one of the most vulnerable organs and can get easily damaged. After trauma of the skin, scarring is likely to occur. However, in some cases and in some individuals, skin trauma results in abnormal or excessive scarring. This thesis describes current modalities for the treatment of abnormal scars. The focus lies firstly on the prevention of hypertrophic scars following acute burn wounds or reconstructive wounds. Secondly, novel treatment modalities for keloid scars are evaluated. Finally, it provides insights in current subjective and objective tools for the measurement of scar characteristics.

Skin

Anatomy

The skin consists of numerous cellular components divided over two layers: a top layer or *epidermis*, which is the visible part of the skin and forms a waterproof, protective barrier (figure 1). The epidermis itself consists of five layers and contains no blood vessels. The lowest (or deepest) layer of the epidermis is called the *stratum basale* or *basal layer*. In the basal layer, new cells are formed through mitosis. From there, cells ascend to the upper layer of the epidermis called the stratum corneum. Around 90% of the cells in the epidermis are keratinocytes, which primarily function as a barrier against the outer environment. Other epidermal cells include: melanocytes (producing pigment), Langerhans cells (immune cells) and Merkel cells (sensible cells). Directly below the epidermis, a thin layer, called the basement membrane, is positioned. The basement membrane anchors the epidermis tightly to the dermis. The second skin layer is called the *dermis* and is located underneath the epidermis. The dermis is a thick, rubbery skin layer that consists of connective tissue and cushions the body from stress and strain. De dermis harbors nerve ends, hair follicles, diverse type of glands, lymphatic vessels and blood vessels. Underneath these layers, subcutaneous fat is positioned.

Wound healing

Despite the extensive capabilities of the skin, it is also one of the most vulnerable organs. When the skin is damaged, cutaneous integrity must be restored to prevent fluid loss, influx of pathogens or physical impairment. Wound healing of the skin is an extremely broad and complex process, which is still not completely understood. Progression from traumatic injury to the formation of a stable scar can be described in four overlapping, but biologically distinct phases to repair the skin: After the *hemostasis*, the *inflammatory* phase is initiated, then the *proliferative* phase and finally the *remodeling* phase.

The *hemostasis* begins directly after a tissue injury and serves to prevent further blood loss by the formation of a blood clot. Thereafter, the *inflammatory* phase begins and various soluble factors (including chemokines and cytokines) are released to attract cells that remove devitalized tissues and prevent invasive infection by pathogens. As inflammation subsides, less inflammatory factors are secreted, marking the start of the proliferative phase.

The *proliferative* phase of wound healing lasts from around day 4 to 21 after wounding. During this phase, extracellular matrix (ECM) is formed in the open wound, serving as a scaffold for structural repair and vascular ingrowth. ECM is mainly formed by fibroblasts. Meanwhile, keratinocytes migrate to restore epithelial continuity (re-epithelialization). Finally, the *remodeling* phase starts from about day 21 and lasts up to 1 year. In this phase the wound will be closed by wound contraction and collagen remodeling.[1,2]

Scarring

In clinical practice, many patients ask: “Will there be a scar?” This question can be answered using the previously discussed knowledge that the skin consists of two layers; the epidermis and the dermis. Although impairment of the epidermis can heal without scar formation, deep dermal or full-thickness (total dermis) wounds will not heal without scarring. Thus, when a full-thickness injury occurs to the skin or an incision is made, there will always be a scar. The question therefore is not whether a scar will form, but what kind of scar will be formed.

Normally, after full-thickness trauma of the skin, a flat linear scar follows leaving an erythematous trace of the original injury that caused it. Over time, most scars will become softer and paler by maturation. Remarkably, fetal skin impairment in utero will heal mainly by regeneration, thus ‘scarless’.[3]

Burn wound scars

Opposed to scars following trauma or surgery, burn wound scars are often not linear, but widespread, frequently covering a significant percentage of the total body surface area. Burn wounds are classified into *three* degrees: *First degree* burn wounds, which only involve the epidermis. These burn wounds will heal within 4-5 days without scarring. With s*econd degree* burn wounds, the total epidermis and the upper part of the dermis are affected resulting in blistering. However, healing will occur within 7-14 days with minimal to no scarring. When both epidermis and dermis are destroyed, a *third degree* burn wound is diagnosed. In this case, wound healing can only occur from the wound edges, often resulting in abnormal scarring.

Abnormal scarring

Successful wound healing requires a delicate balance between ECM deposition and degradation.[4,5] However, in predisposed patients, certain groups (e.g., people of African and Asian descent) or burn victims, the normal wound healing process may be aberrant.[2,6] In these individuals, wound healing leads to increased collagen deposition and decreased collagen breakdown, resulting in excessive scar formation. This excessive scarring may be the result of pathological persistence of wound healing signals or an inadequate down regulation of tissue repair.[4,7] Aberrations in each of the phases of wound healing may contribute to this process.[2,7]

On cellular level, fibroblasts are key players in the deposition of excessive scar tissue. Their activity and proliferation is affected by a multitude of factors. Excessive scarring eventually leads to the formation of two types of scars: hypertrophic scars and keloid scars.

Figure 2. Upper Left: Keloid scar (frontal and transverse angle), originated from a small incision and grew beyond the boundaries of the orginal lesion. Below Left: Hypertrophic scar originated from *surgery*. Right: Hypertrophic scarring following *burn wounds.*

Hypertrophic scars

Hypertrophic scars are raised scars, characterized by inflexible, red or hyperpigmented colored tissue with an irregular surface texture. Besides this aesthetic burden, they can cause physical complaints such as pain and itching, impairing the quality of life of the patient.[2,8] Hypertrophic scars formation can result from surgery or any trauma of the skin. They are often seen following large burn traumas (figure 2).

Prevention

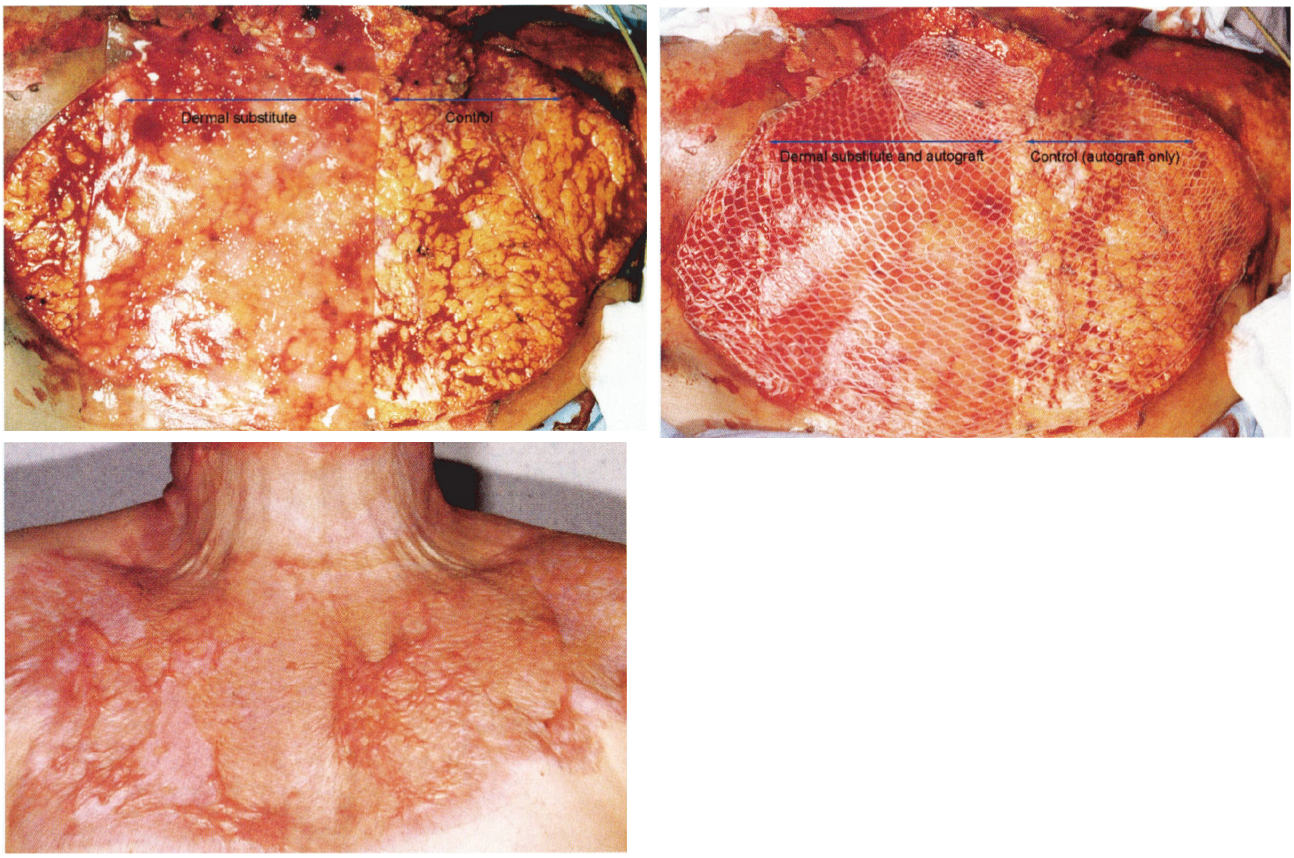
Prevention of hypertrophic scars formation is especially sought for large reconstructive or burn wounds. Burn wound scars are often not linear, but widespread and can cover a significant percentage of the total body surface area. In the case of third degree burn wounds (full thickness wounds), wound healing can only occur from the wound edges. In large third degree burn wounds healing from the wound edges will not occur or will take a long time, resulting in severe scarring and wound contractures. It is therefore important to cover the burned area. Currently, the golden standard for the treatment of deep second degree or third degree burn wounds is the autologous split thickness skin graft. This split thickness graft is harvested from an unaffected site of the body, mostly the upper legs. The graft is then processed by a skin mesher, which makes apertures (holes) onto the graft, allowing the graft to expand up to nine times its size (figure 3). In this way, large areas can be covered. Since the epidermis and only a small part of the dermis are used to create the graft, the donor site will heal without severe scarring.

Although the burn wound is protected by the skin graft from infections and the graft improves the wound healing process, it often heals with severe hypertrophic scarring and major wound contractures. Hereby, the interstices (apertures) of the grafts, which heal by epithelialization from the grafts margins, are particularly prone to hypertrophic scarring. Most of this scarring and contraction is due to the lack of dermal components in the split thickness graft. Therefore, dermal substitutes were introduced: By replacing the dermal layer of the skin with a dermal substitute, scar quality improves, resulting in more elasticity and less hypertrophy of the skin.[9] See figure 3. Dermal substitutes are available in three different materials: natural biologic materials, constructed biological materials and synthetic materials. The biological background of these dermal substitutes is discussed in **chapter 2**. Although good outcomes were reported with the use of dermal substitutes, no long-term results have been published.[9,10] Therefore, we conducted a 12-year follow-up with the use of a dermal substitute, as discussed in **Chapter 3** of this thesis.

Treatment

Hypertrophic scars are self-limiting and can regress over time by flattening and fading. Hypertrophic scars can be treated using surgical excision or nonsurgical by using compression therapy, laser treatment, gel sheeting or intralesional corticosteroid injection.[2,11]

Figure 3. Upper Left: Dermal substitute placed on the right side of the chest. Upper Right: On top, a split thickness skin graft was placed. The graft uses apertures to increase its cover area. Below Left: The appearance of the scar with the dermal substitute (right side) seems smoother, with less hypertrophic scarring compared to the left side



Keloid scars

The archaeologist Edwin Smith discovered the first description of keloid scars in 1930, when he translated a papyrus from ancient Egypt detailing surgery at that time. The actual term keloid was introduced by French Dermatologist Baron Jean Louis Alibert in 1806.[12] Alibert used the term “Cheloide”, derived from the Greek word khēlē, meaning crab’s claw.

Keloid scars differ from hypertrophic scars in numerous ways. *Firstly*, they differ in their growth pattern: Hypertrophic scars are defined as a fibroproliferative disorder of the skin that does not grow beyond the boundaries of the original wound.[11] In contrast, keloid scars grow beyond the boundaries of the original wound or have an unrecognized origin.[11] See figure 2. *Secondly*, pathologists distinguish keloids from hypertrophic scars histologically on the basis of thick (hyalinizing) collagen bundles, which are absent in hypertrophic scars.[11] Also, compared to hypertrophic scars, keloidal fibroblasts show a greater response to signals stimulating scar formation: For example, keloidal fibroblasts respond to exogenous TGF-β with a much greater collagen production than hypertrophic scar fibroblasts. *Thirdly*, as opposed to hypertrophic scars, which can occur at any site of the body, keloid scars are more prone to develop on specific sites, including the chest, shoulders, earlobes, upper arms and cheeks.[2,7] Finally, keloid scars do not regress over time but persist or even evolve, causing physical complaints of pain and pruritis, as well as posing an aesthetic burden.[4,7,13] This makes treatment difficult, with high recurrence rates as the main issue.[2] Since surgical excision is associated with high recurrence rates (45 to 100 percent), other treatment modalities have been developed.

Treatment

Treatment usually starts with intralesional undiluted corticosteroid injections (Kenacort-A '40', Bristol-Myers Squibb bv). Often, multiple sessions are required and caution should be made not to overtreat the scar resulting in atrophy of the skin and subcutaneous tissue. If non-surgical treatment modalities are unsuccessful the next step is usually cryotherapy.

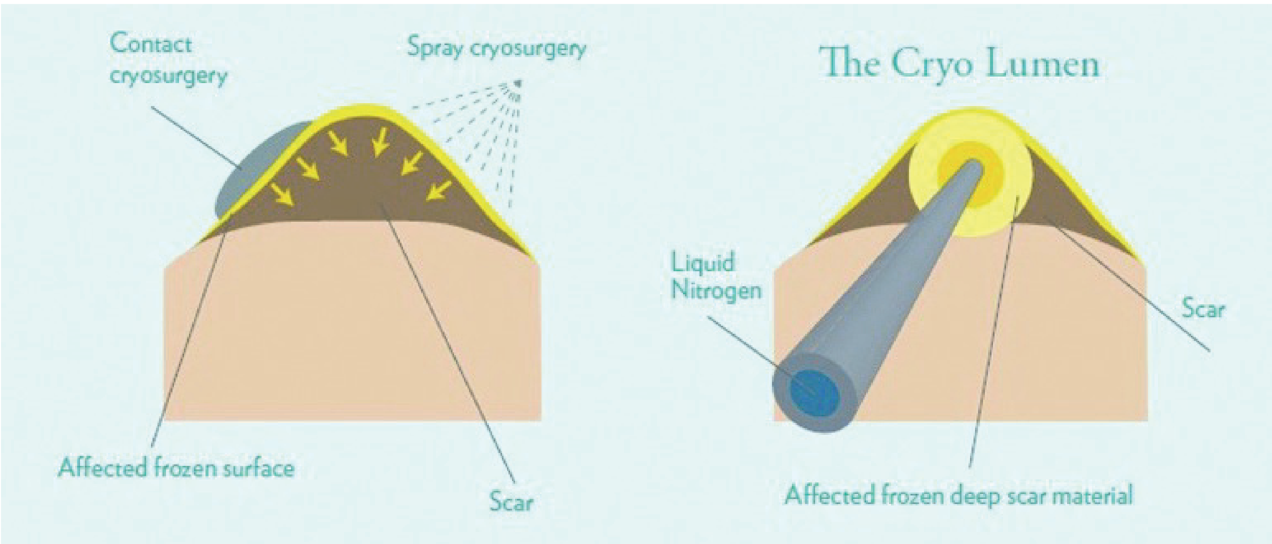
Intralesional cryotherapy

Cryotherapy, also called cryosurgery or cryoablation, is a well-established therapy for destruction of tissues.[14] Even Before Christ (BC), mankind was aware of the beneficial effects of cold. During the 5th century BC, Hippocrates already described the use of cold to control hemorrhages and reducing swelling. Today, cryotherapy is even used in the oncological treatment of kidney or prostate cancer by minimally invasive techniques.[15]

For decades, liquid nitrogen has been applied using external techniques to treat keloid scars. However, multiple side effects such as hypopigmentation, blistering, delayed healing and infection were reported.[16,17] Another problem was the difficulty of reaching deeper dermal sections of the scar, which resulted in high recurrence rates and limited the use of cryotherapy to small scars only.[16,17] In 1993, Weshahy devised a solution by introducing a new method that applied liquid nitrogen intralesionally (into the lesion) with a hollow needle: Intralesional (IL) cryotherapy.[18] By using this hollow needle, a cryogen could be administered directly into the deeper dermis, resulting in more volume decrease and less recurrence. Also, the epithelium was affected less, thus reducing blistering and hypopigmentation. See figure 4. In a systematic review, promising results following IL cryotherapy were seen, however only small Caucasian patient populations were included (**chapter 4**). Since keloid incidence and rates of recurrence and pigmentation problems are higher in Afro-American individuals[19,20], a study including patients of all different skin types was desirable. Therefore, we evaluated IL cryotherapy in a prospective multicenter study with a patient population including all skin types (**chapter 5**).

In this study we found a significant volume reduction of the scar and alleviated complaints of pain and pruritus. However, we also experienced a limited and unreliable freezing capacity of the liquid nitrogen-based device, occasionally resulting in elongated freezing times and even in dysfunctional treatments. This was not only undesirable from a therapeutic point of view, but also resulted in traumatic experiences for the patients, since in those cases (local) anesthesia had already been administered. Therefore, IL cryotherapy, in another study, was tested with a different system based on argon gas (**chapter 6**). This argon gas based system requires no precooling (as opposed to the available liquid nitrogen devices) and offers controlled and accurate freezing. As a result, no dysfunctional treatments were seen and less recurring scars were observed. To gain insight into the working mechanism of both systems (argon gas-based and liquid nitrogen-based), we designed an experimental study to investigate the thermal behaviour of these cryosystems (**chapter 7**).

Figure 4. Left: Contact or surface cryotherapy, hereby affecting mostly the surface epithelium. Right: Intralesional cryotherapy, which targets directly the core of the keloid, while at the surface, cells including melanocytes are much less affected. (Source; prof y. Har-Shai)



Surgical excision with adjuvant radiation therapy

Surgical excision offers a final treatment option, if all other non-surgical treatment modalities have proven unsuccessful. However, surgical excision alone is associated with high recurrence rates (45 to 100 percent).[2] Therefore, adjuvant therapy is required following scar excision.[2,11] According to the international advisory panel on scar management, surgical excision with post-operative radiation therapy is considered the most efficacious treatment.[21] Radiation therapy for treatment of keloid scars was first described by Sequira in 1909.[22] Traditionally, it was applied *externally* using a variety of devices.[23] Although good results were achieved, external radiation therapy requires a relatively high irradiation dose due to the large distance between the radiation source and the scar. Also, the surrounding healthy skin is unnecessarily exposed to radiation.[24] To solve these problems, Malaker et al. introduced a technique called *brachytherapy* (also called *interstitial* or *internal radiation)* in 1976 .[24] Nowadays, it is available as low-dose-rate (LDR) or high-dose-rate (HDR) brachytherapy. With both methods, a hollow catheter is incorporated in the surgical lesion after excision of the scar, through which a radioactive source is directed. See figure 5. In this way, irradiation is effectively localized from inside the lesion, only targeting the desired area.[24] With LDR brachytherapy, a low dose radioactive source is used and removed after typically 20-72 hours.[25] In contrast, with HDR brachytherapy, a high radioactive source is applied for a short period of 5-10 minutes.[26] Due to the short treatment time, HDR brachytherapy is an out-patient procedure enhancing patient convenience, whereas LDR brachytherapy requires hospitalization.

Figure 5. Placement of a catheter used for High-Dose-Rate Brachytherapy after excision of a keloid scar

In chapter 8, we discuss these treatment modalities further, based on our study in which we sought for the optimal radiation protocol. In chapter 9, we present our 10 year experience with a high-dose-rate brachytherapy procedure. In this study, a protocol was used with the lowest total radiation dosage known in literature. Finally, in chapter 10, a novel method for large keloid scars is discussed.

Current scar research

Modern scar research requires quantification of scar characteristics. In other words, we want to measure what we see when looking at scars or residual scars before and after treatment. Importantly, this will also allow for comparisons to be made between studies to ultimately identify the best treatment modality. Therefore, reliable and validated evaluation instruments have been developed to obtain objective and subjective outcome parameters.[27] In the studies presented in this thesis modern subjective and objective measurement devices were used.

For subjective evaluation of scars, the 'Patient and Observer Scar Assessment Scale' is a reliable and valid scar assessment scale tested for hypertrophic and keloid scars.[28–30] Both the patient and observer scale consist of six items that are scored using a 10-step score, in which 10 reflects 'worst scar imaginable' and 1 indicates 'normal skin'. For objective scar assessment, like *scar elasticity* we used the Cutometer (Courage and Khazaka GmbH, Cologne, Germany).[31] The Cutometer measures the skin elasticity using negative pressure, also called the suction method. The resistance of the skin to the negative pressure and its ability to return into its original position are displayed as curves during the measurement. Elasticity parameters, such as skin extension, pliability, elasticity, retraction and viscoelasticity are determined based on the skin's resistance to negative pressure and its ability to return to its original state. *Skin colour* is important because redness or (de)pigmentation of the skin is considered a very disturbing end result.[32,33] Scar pigmentation (melanin index) or scar redness (erythema index) can be objectively determined by using the Dermaspectrometer (Cortex Technology, Hadsund, Denmark).

The apparatus uses red and green light reflection of the skin, which are important parameters for the skin color changes. *Scar surface roughness* is of particular value when evaluating burn scar treatments. Scar roughness is measured using the Phaseshift Rapid in Vivo Measurement of Skin or P.R.I.M.O.S. (GFMesstechnik GmbH, Berlin, Germany).[27] This device produces a three-dimensional image of the surface of the skin. Finally, *scar volume* is of importance when measuring effectiveness of keloid scar treatments. In this thesis, the scar volume was determined by creating a mold of the scar with dental putty (Cavex CA37, Alginate impression material, CAVEX Holland, the Netherlands).

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Part I
Dermal substitutes for treatment of acute burns and reconstructive wounds

Chapter 2

Biological background of dermal substitutes

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Martijn van der Wal
Michiel van Leeuwen
Magda Ulrich
Esther Middelkoop

Burns 2010;36(3):305-21.

Abstract

Dermal substitutes are of major importance in treating full thickness skin defects, both in acute and chronic wounds. In this review we will outline specific requirements of three classes of dermal substitutes:

- natural biological materials, with a more or less intact extracellular matrix structure;
- constructed biological materials, composed of specific biological components; and
- synthetic substitutes, which can be synthesized on demand and can be modulated for specific purposes.

Biological and clinical requirements will be translated to composition, physical structure, immunological properties and cell–matrix interactions of the various materials.

Important properties like pore size, cell adhesion sites (e.g. RGD (Arg-Gly-Asp) sequences), crosslinking, degradability and the presence of a basement membrane will be discussed for each of the different classes of materials.

Introduction

Improved healthcare raised new challenges in wound care. Life expectancy has increased to such an extent that chronic wounds associated with aging and diabetes have become more significant.[1] In burns, technological developments in the intensive care treatment as well as the development of dedicated burn centers increased the survival rate of severely burned patients considerably. Excision of the burned area and grafting with autologous split thickness grafts became the gold standard, and still is today. However, with the introduction of new treatments new problems became apparent, such as the severe scarring which occurred after grafting with meshed split thickness skin grafts. The interstices of the grafts, which healed by epithelialization from the graft margins, were particularly prone to hypertrophic scarring. Furthermore, the need for donor sites sometimes exceeded the available unburned skin. This has encouraged the development of skin replacement materials.[2]

The problem of lack of donor sites was tackled by the development of cultured epithelial autografts (CEAs). In first instance, the literature reports on clinical application were very enthusiastic.[3-7] However, problems associated with the use of Cultured epithelial autografts (CEAs) for large and deep burn wounds were the variable take of the grafts and long lasting fragility of the skin after healing.[8-10] The latter found its origin in the poor regeneration of the basement membrane, the absence of anchoring fibrils, and lack of dermal tissue which is now thought to be responsible for the reduced quality of the healed skin (or scar) after application of cultured epithelial autografts (CEAs).[11-13] Other problems associated with the use of cultured cells were the necessity to use animal-derived cells and/or proteins in the culture system, which potentially could allow disease transmission of viruses or prions from animal to humans, the high costs associated with cell cultures, difficulties in handling and transfer, and the time span needed to produce enough cultured cells.[12-15]

The lack of dermal tissue in full thickness wounds and the poor quality of the scars after treatment with split thickness autografts or cultured epithelial grafts which contain little or no dermal component respectively, initiated the development of dermal substitutes.[12,16]

Despite the widespread efforts, the clinical application of dermal substitutes did not in every case provide the promising results which were demonstrated with animal models.[17] Margolis stated that “in general, very few new experimental treatments for chronic wounds have performed as well in the clinical setting as in the preclinical lab setting”.

Furthermore, there is a lack of biological background information on the design and use of different types of materials and their influence on the surrounding tissue.

In this review we outline the biological background of three classes of dermal substitutes: (1) substitutes with a complete extracellular matrix (ECM) architecture originating from human or animal sources such as Alloderm[1,18] and Oasis[1,19]; (2) substitutes made of biological components such as Integra[1,20] and Matriderm[1,21]; (3) substitutes which are composed of synthetic materials such as Dermagraft[1,22] and Polyactive.[1,23] We relate several characteristics to clinical requirements. In addition, we discuss the available dermal substitutes as well as in vitro, in vivo and clinical test systems.

Functional requirements of dermal substitutes

Some general principles for adequate function of dermal substitutes can be formulated (table 1). Translating these clinical requirements into mechanical and physical properties of the material is more complicated.

Table 1. General principles for adequate function of dermal substitutes

Basic principles dermal substitutes	
A	Protect the wound from infection and loss of fluid
B	Provide a stable and biodegradable template for the synthesis of neodermal tissue
C	Either host or enable the influx of cells that will function as dermal cells, producing dermal tissue rather than scar tissue
D	Allow ease of handling and resist tear forces

A. Protecting the wound from infection and fluid loss can be achieved by providing the dermal substitute with an impermeable wound cover. Over the years two strategies have been developed to achieve this goal. The first is to achieve temporary wound coverage by applying a dermal substitute to which an impermeable cover is attached, usually a silicone layer. After adequate ingrowth of vasculature into the dermal tissue the silicone layer is removed and replaced by an autologous split-skin graft. This procedure is now known as the two-step procedure. The ingrowth of vessels may take up to three weeks, during which period there is still a threat of wound infection.[20,24,25] This is the main drawback of this approach. Therefore, a second strategy has been developed. This method is based on a one-step procedure: immediately after debridement the dermal substitute is placed in the wound and covered by an autologous split-skin graft. This method provides earlier wound closure, however, graft survival may be hampered by the presence of a non-vascularized dermal substitute in the wound.[26] Therefore, not all materials will allow application in a one-step procedure. The possibilities to apply this technique will depend on other material characteristics such as pore size and influx of cells which will be discussed below.

   A temporary wound cover could also be provided separate from the substitute itself, by application of an adequate wound dressing material. Therefore, the temporary covering does not need to be an intrinsic property of the dermal substitute.[27,28]

B. Stability, biodegradation and immunocompatibility are key issues for design and function of a dermal substitute. The material should preferably represent a temporary replacement of the lost dermal tissue, and provide an adequate environment for the formation of new and functional dermal tissue. Although optimal degradation time of a scaffold is unknown, general statements on the time the scaffold must remain in the wound environment can be made.

If stability of dermal substitute materials is too low (disintegration within days), the template function of the substitute will not be fulfilled. In humans, the proliferation/ migration phase of the wound healing process will generally take up to three weeks. During at least this period, the dermal substitute should provide a temporary three-dimensional structure to allow ingrowth of blood vessels, fibroblasts and coverage by epithelial cells. Stability of biomaterials can generally be increased by physical and/ or chemical crosslinking of the material.[5,29-31] Care should be taken in application of such methods, since the crosslinking agents may seriously affect wound healing, e.g. by toxicity due to remnants of crosslinking agents or induction of a foreign body response.

Biodegradation should preferably take place after this period, and by such a process that no massive foreign body reaction is induced. This would increase the inflammatory response, which is usually already high especially in burn wounds, and which is associated with profound scarring.

Furthermore, the dermal substitute should be composed of immunocompatible material, to avoid immunoreactive processes.

C. Host or enable the influx of cells that will function as dermal cells.

In general, cell migration and cell function are influenced by the composition, pore size and degradability of the dermal substitute.[32–34]

Hosting of cells would limit the requirements for influx of the hosted cells, usually fibroblasts. However, the necessity for influx of endothelial cells is undiminished. The concept of incorporated cells may be very attractive from a scientific and functional point of view, the complications in production and availability of such a substitute are huge. Choice of cell type (allogeneic versus autologous[35]), design as ready to use or custom made, complications associated with cell culture procedures (costs, potential disease transmission) are some of the difficulties associated with tissue engineered or living skin substitutes.[13,14]

D. Finally, the surgeon should be able to place the dermal substitute and the material should be able to resist a certain level of shear forces, especially when applied on difficult areas such as back, buttocks, or joint areas. Air pockets and dead space between the dermal substitute and wound bed should be avoided.[36]

Classes of dermal substitutes

Natural biological materials

Natural biological materials consist of human or porcine cadaver tissue which is treated to produce an acellular scaffold for use as a dermal substitute. The main advantages of biological materials as dermal substitutes are that the scaffold's composition and organization is highly similar to native dermis and that parts of the basement membrane may be conserved. There are also some major drawbacks. The allogeneic nature of these structures may give rise to rejection due to cell remnants which are often difficult to remove. For this reason biological materials are often used as temporary biological dressings rather than permanent dermal substitutes. Furthermore, natural biological materials derived from cadaver material can possibly also transmit diseases to the recipient.

Cadaver skin has some excellent qualities as a biological dressing but is sloughed off 10-21 days after application[37] because remnants of donor cells elicit an immune response from the recipient.[38] In order to use this skin as a functional dermal substitute all immunogenic factors that could lead to rejection of the donor material must be thoroughly removed, while the skins native structure and composition must remain intact. The methods used to reach these goals generally have contrary effects; extremely aggressive removal of immunogenic components may destroy the structure and composition of the material, whereas more gentle treatments may cause the material to remain immunogenic.[39] The group of MacNeil experimented with different methods of removing all cellular remnants from cadaver dermis. Their research demonstrated that aggressive sterilization methods like ethylene oxide or γ-irradiation induced structural changes in the dermis, while sterilization with glycerol gave a more natural dermal structure.[40] Retaining the natural structure of the dermis is thought to be vital for these materials, as the natural basket weave patterning of the dermal substitute may promote the deposition of a naturally oriented neo-dermis by the infiltrating fibroblasts, and to inhibit the production of abnormal collagen patterns typical for scars.

These materials usually contain parts of the native basement membrane on the papillary side. Several studies showed that the presence of a basement membrane greatly improves the adherence, outgrowth and differentiation of keratinocytes[41] and that this effect is associated with the presence of laminin and collagen IV in the basement membrane.[42] MacNeil et al. also demonstrated that the procedure used for de-epidermization was vital in retaining the basement membrane of cadaver skin. While enzymatic de-epidermization resulted in the removal of important basement membrane molecules, gentle de-epidermization in (phosphate buffered) saline generally resulted in a more intact basement membrane which subsequently produced a better epidermis when used to construct a full-skin equivalent.[40]

Several procedures for de-epidermization and de-cellularization are currently in use for the production of dermal substitutes from cadaver skin. Alloderm1 is one of the oldest and most used and consists of NaCl–SDS treated dermis that shows retention of the basement membrane and good immunogenic properties in vitro and in animal studies.[43,44] Clinical studies have shown it to be useful as a dermal substitute although the graft take of split-skin grafts on top of Alloderm1 in a one-step procedure was somewhat lower than split-skin graft applied alone.[18] A more recent addition to this family of substitutes is Glyaderm, a NaOH treated glycerol preserved cadaver skin.[39] Experiments performed by Pirayesh et al. (manuscript in preparation) indicated that the take rate of split-skin grafts was reduced when applied as a one-step procedure on top of Glyaderm or Alloderm[1] in a porcine full thickness wound model. Application of Glyaderm in a two-step procedure, allowing the dermal substitute to become well vascularized before split-skin application, resulted in better take rates and reduced wound contraction compared to control wounds treated with only split-skin grafts. Sahota et al.[45] reported on the problematic take rate of natural substitutes in challenging wound beds as a result of poor vascularization. They investigated the penetration of endothelial cells into full-skin equivalents constructed from acellular cadaver skin to gain insight into the vascularization of skin grafts. Their findings indicated that migration of endothelial cells into the cadaver dermis was rather slow and that migration increased where the dermis was damaged. The density of the collagen fibers in this class of dermal substitutes could present problems for rapid revascularization in vivo.

Constructed biological materials

A second class of dermal substitutes consists of purified biological molecules formed into a dermal substitute by means of lyophilisation, which can be supplemented with glycosaminoglycans and also crosslinked in order to control its properties.[29] Collagen is often used as the main component. Different freeze drying procedures can be used to control aspects such as pore size and pore interconnectivity during scaffold production. The artificial nature of these products results in some distinct advantages and disadvantages compared to natural biological structures. The use of purified skin components allows the designer to select materials that will not elicit a rejection response. Also the composition and properties of the product can be much more precisely controlled and in theory many beneficial growth factors and matrix components can be added to the product. However, at present the knowledge of what should be incorporated in these materials and what should be avoided is not sufficiently present to take full advantage of the potential of these materials. Furthermore, these materials often lack a basement membrane and their architecture does not resemble native skin. Well known examples are Integra[1,20,46,47] and Matriderm[1,21,48] which are used in the treatment of burns.

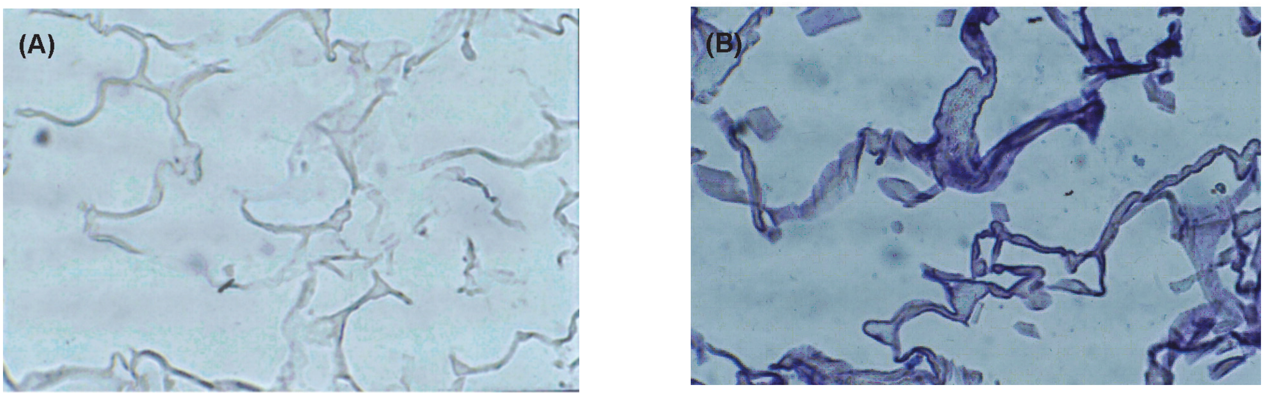
The first major benefit of this class of materials is that the rejection response can be avoided by careful selection of the proteins used to construct the dermal substitute, generally collagen. The nature and extent of collagens immunogenicity remains somewhat unclear. Evidence has been published that the telopeptides located on the ends of the tri-helical collagen molecule may provoke an immune response and that removal of such peptides produces nonimmunogenic “atelocollagen” which is better suited for the construction of biomaterials.[49] However, one of the most recent reviews published on this subject questioned the relevance of collagen immunogenicity and the supposed benefits produced by removal of telopeptides. The lack of rejection problems observed in (telopeptide containing) collagen based substitutes strongly suggests that any immunogenicity that collagen may exhibit is not problematic in its application in wound healing.[50]

Cells attach to the extracellular matrix (ECM) by specific cell surface receptors, the integrins. Many integrins recognize specific amino acid sequences, e.g. of fibronectin and vitronectin, the RGD sequences. The interaction of integrins with these sequences allows the cells to attach to these fibres. Although collagen and laminin also contain RGD sequences, these are inaccessible to cells in native skin. The intracellular parts of integrins are linked to the cytoskeleton and also function as signal transducers whereby the extracellular matrix (ECM) can guide cell behaviour.[51] Furthermore, fibroblasts and keratinocytes can secrete matrix metalloproteinases (MMPs) which are able to degrade many extracellular matrix (ECM) components. The combined action of integrins and matrix metalloproteinases (MMPS) allows cells to migrate through these materials and remodel them. Constructed biological dermal substitutes generally consist of 1-2% solid content. As a result of the low solid content and high biocompatibility of these materials, they are relatively unstable. To allow the material to remain in the wound area long enough to exert its effects on the wound healing process, it often requires crosslinking to reinforce its stability.

Although crosslinking can be used to improve matrix longevity in the wound area, the crosslinking can also have detrimental effects on the wound healing process. Cross-linking materials may give rise to unfavourable reactions in the wound. De Vries demonstrated that in a porcine full-thickness model a glutaraldehyde crosslinked collagen matrix induced a foreign body response (FBR) compared with non-crosslinked collagen matrix. This affected dermis formation, split-skin graft take and contraction outcome parameters in an unfavourable way, despite the faster degradation rate of the non-crosslinked materials.[52] In addition, chemical cross-linking can often produce degradation products that are detrimental to cell survival. In vitro experiments with several types of chemically crosslinked collagen matrices showed decreased fibroblast survival and proliferation.[53] Another concern with regard to crosslinking is increased matrix rigidity and the effect this may have on the phenotype of fibroblasts. Increased extracellular matrix (ECM) rigidity is an important factor in myofibroblast differentiation.[54–56] Although myofibroblasts play an important role in the wound healing process, excessive presence of myofibroblasts can lead to increased contraction and collagen deposition resulting in hypertrophic scaring.[57,58]

Alternatively, degradation of the collagen main structure can be reduced by addition of extracellular matrix (ECM) components that protect it from matrix metalloproteinase (MMP) degradation. During the development of their artificial skin Yannas et al. found that addition of glycosaminoglycans (GAGs) such as chondroitin 6-sulphate, chondroitin 4-sulphate, dermatan sulphate, heparin and heparin sulphate to collagen scaffolds could increase their resistance to collagenases. This decreased the need for excessive crosslinking. Using the glycosaminoglycans (GAGs) also allowed them to control certain mechanical properties and pore sizes of their scaffolds.[36] De Vries et al. demonstrated that coating the collagen fibres in dermal substitutes with fibronectin, hyaluronic acid or elastin (figure 1)[59] could stabilize these matrices in a porcine full-thickness wound model. The material remained present for up to 4 weeks while untreated collagen matrices were degraded within 1-2 weeks.[60] However the effects of these additives on the wound healing process varied; hyaluronic acid and fibronectin did not improve wound healing and had some adverse effects on granulation tissue development and re-epithelialization, whereas elastin coated collagen matrices reduced granulation tissue formation, fibrosis and contraction and stimulated collagen deposition by fibroblasts.

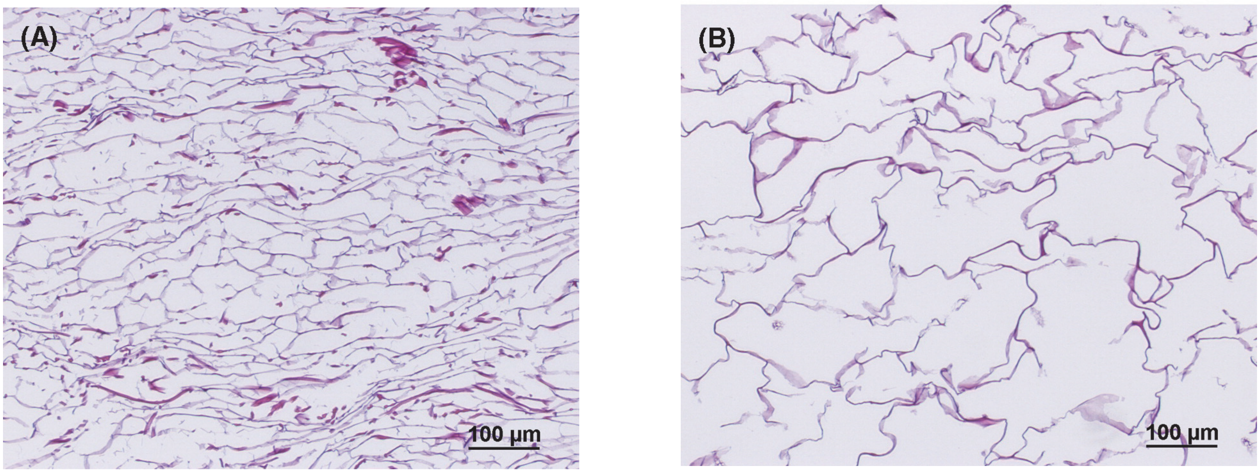
Figure 1. Non-crosslinked collagen matrix (a) and non-crosslinked elastin–matrix (b) stained with anti-elastin antibodies



Matrix additives also play an important role in the vascularization of dermal substitutes. Collagen/chondroitin-6-sulphate scaffolds like Integra[1] require a two-step surgical procedure to allow vascularization of the scaffold prior to split-skin grafting. Simultaneous application of the matrix and a split-skin graft would generally result in graft loss, as it could take more than three weeks for the dermal substitute to become fully vascularized.[61,62] Studies by Lamme et al. demonstrated that collagen/elastin scaffolds showed increased vascularization one week post-wounding, compared to split-skin grafted wounds in a porcine excisional wound model.[63] This increased rate of vascularization may explain why Matriderm[1] can sustain a split-skin graft when applied in a one-step procedure.[26]

This difference in vascularization is explained by the different actions of elastin and chondroitin-sulphate. While chondroitin-6-sulphate (chondroitin-sulphate A) had anti-angiogenic properties when tested in a chorioallantoic membrane (CAM) assay[64,65], elastin and elastin derived peptides on the other hand promoted angiogenesis in a chorioallantoic membrane (CAM) assay.[66] Elastin derived peptides were shown to function as chemoattractants to vascular smooth muscle cells.[67] Pore size did not seem to limit vascularization in this case, as Integra[1] scaffolds have a larger pore size than Matriderm[1] scaffolds (figure 2).

Figure 2. HE staining of Matriderm® (A) and Integra® (B).



Synthetic substitutes

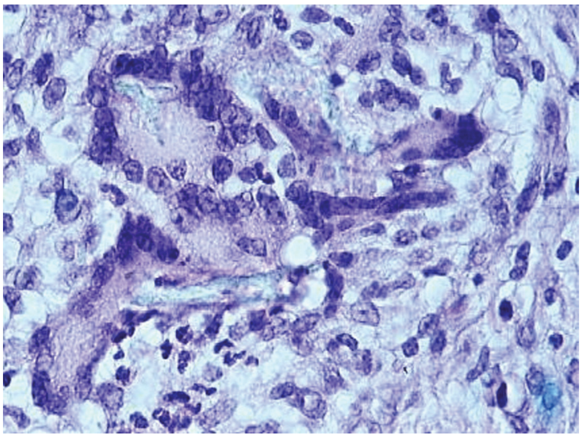
Similar to the biological constructs, dermal substitutes can be constructed out of non-biological molecules and polymers not present in normal skin. Although this provides the producers with even more control over the precise composition of their product, the use of non-biological components can be problematic when trying to produce a biologically compatible material. Although only few are presently in use, a substantial number of synthetic substitutes are currently undergoing in vitro or animal testing to assess their potential use as dermal substitutes.[68,69] Instead of describing a large and highly diverse number of materials and production methods, we will constrain ourselves to some elemental issues that need to be kept in mind when dealing with synthetic dermal substitutes.

Fibroblasts and other cells that need to invade or cover the material need to be offered binding locations and chemotactic signals in the material to allow cell migration and guide cell function. Although many cell types are able to adhere to synthetic materials such as the plastic used in traditional cell culture techniques, the interactions of cells with these materials is distinctly different from those in natural extracellular matrix (ECM). The architecture and composition of the substrates can have an impact on cell-adherence, migration, stability, signaling, cell function, etc.[70,71] The fact that synthetic materials generally only offer binding sites not occurring in native tissue may be problematic in the biological functioning of these materials as dermal substitutes. To overcome these problems, biomimetic protein sequences can be integrated in these matrices to facilitate the movement of cells. In recent years RGD sequences have been incorporated into synthetic materials in order to improve their use in tissue engineering applications.[72,73] Several studies have demonstrated that incorporating these RGD sequences in self-assembling hydro-gels facilitates the migration and persistence of fibroblasts in these materials and resulted in more natural cell morphology and increased cell–matrix interactions such as contraction.[74,75]

Invading cells also must be able to degrade the material. Matrix degradation plays an important role in two processes vital to the wound healing process; cell migration and foreign body response (FBR). Concerning cell migration, Lutolf et al. showed how incorporation of matrix metalloproteinase (MMP) degradable sequences into hydrogels increased the cel invasion rate of fibroblasts compared to controls not containing the matrix metalloproteinase (MMP) degradable sequences.[76] Furthermore, Raeber et al. demonstrated that inhibiting matrix metalloproteinase (MMP) activity inhibited cell migration through these gels.[77] The extent to which migration was inhibited in response to matrix metalloproteinase (MMP) inhibition appeared to be highly dependent upon the pore size of the material. In materials with very small pores, such as the hydrogels used in these studies (pore size of ∼25 nm), matrix metalloproteinase (MMP) inhibition resulted in practically complete inhibition of migration. When pore sizes were increased cells appeared to be less dependant on matrix metalloproteinases (MMPs) for their movements through the material. Fibrin gels (pore sizes 0.1–1 μm) showed a decrease in migration in response to matrix metalloproteinase (MMP) inhibition, however to a much smaller extent than in hydrogels. When pore sizes were increased even more by using collagen scaffolds (pore sizes 1–10 μm), migration was unaffected by matrix metalloproteinase (MMP) inhibition, suggesting that large pore sizes allow fibroblasts to migrate without needing proteolysis.[78]

With regard to foreign body response (FBR), reduced matrix degradability is associated with increased foreign body response (FBR) against implanted dermal substitutes.[52,79] Materials that remain in the wound area for an extended period of time, or are degraded into components that cannot readily be metabolized, may cause the body's immune system to initiate a foreign body response (FBR). This reaction will attempt to remove the implanted material by encapsulation and the actions of fused macrophages called foreign body giant cells (FBGC) (figure 3).

Figure 3. Foreign body giant cells (FBCG) in a porcine wound model

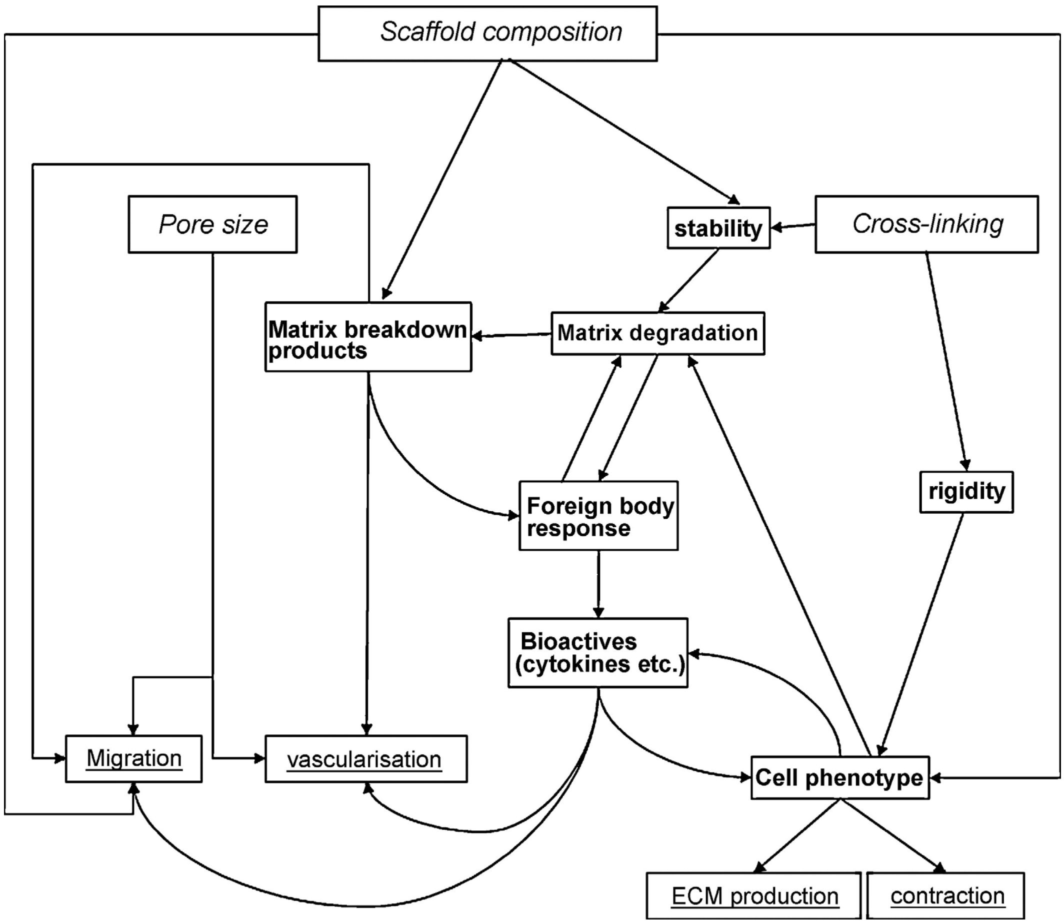


The resulting release of bioreactive and aggressive degrading agents from macrophages and foreign body giant cells (FBGC) involved in the foreign body response (FBR) can adversely affect many aspects of the wound healing process. They can interfere with normal cell communication through the secretion of inflammatory mediators and cytokines and even alter the extracellular matrix (ECM) emodeling process by secreting matrix metalloproteinases (MMPs) and MMP inhibitors (TIMPs).[80] Since synthetic dermal substitutes lack native cell interaction and degradation sites and consist of non-biological polymers, their application in a wound site is more likely to result in this type of response than natural materials. A study by De Vries demonstrated that foreign body response (FBR) is indeed a problem for polyether urethane and polyglactin in a porcine full thickness wound model.[52] An overview of cell-matrix interactions in dermal substitutes is provided in figure 4.

Testing of dermal substitutes

Before a potential dermal substitute is applied in the clinic it should be thoroughly tested to exclude any materials with no positive, or even detrimental effects on wound healing from ever reaching the animal or clinical testing stages. In the discussion of the different classes of dermal substitutes we have already mentioned many general and specific properties of dermal substitutes that need to be controlled to ensure its beneficial function in the wound healing process. In this section, we will discuss several in vitro and in vivo methods of assessing the usefulness of dermal substitutes prior to clinical use and end with a discussion of the clinical evaluation methods.

Figure 4. Overview of cell–matrix interactions in dermal substitutes. Italics: matrix properties, Bold: intermediary processes, Underline: outcome parameters.



In vitro testing

It is important to realize the limitations of in vitro testing of biomaterials; although the direct interaction between matrix and cells can be assessed it must be kept in mind that the actual wound environment is infinitely more complex than the environment inside a Petri dish.

The healing wound is populated by a multitude of different cell types secreting an array of cytokines and other compounds in their attempt to regulate the healing process. Although in vitro models have been developed that might allow a realistic approximation of the wound environment[81], they still lack important aspects such as the influence of the immune system on the healing wound. Currently the primary uses of in vitro tests consist of evaluating materials for cytotoxicity and investigating certain cell matrix interactions which are considered to be beneficial in the wound healing process.

Fibroblasts are the main cell type responsible for the maintenance of the dermis and therefore the most important cell type that must be considered for in vitro testing of dermal substitute materials. Dermal fibroblasts can be cultured and applied to dermal substitutes quite easily and aspects such as cell migration, proliferation and contraction can be readily assessed. The characteristics of a material, based on pore sizes, integrin binding and proteolytic degradation sites, as discussed above, can be evaluated on the basis of such experiments. A more complicated matter is the differentiation of fibroblasts into myofibroblasts, which is commonly associated with increased wound contraction and hypertrophic scar formation.[57,58] Although the effect of the matrix on myofibroblast differentiation can be readily assessed by observing the contraction of the material during the culture period and staining the fibroblasts for alpha-smooth muscle actin (αSMA) afterwards, an important aspect of myofibroblast differentiation is the rigidity of the cell substrate.[54,56,82] The design of the experiment must consider this aspect when choosing whether and if so, how the scaffold will be restrained during the experiment.

An in vitro model of burn wound healing in human skin did not induce the differentiation of myofibroblasts.[81] This shows that the presence of complete human skin alone is not sufficient to accurately model the in vivo situation.

Keratinocytes are another important cell type that interacts with a dermal substitute in a wound healing situation and can be investigated in cell culture systems. The rapid reestablishment of the epidermal barrier function after a skin defect is vital for preventing wound infection and restoring homeostasis to the wound area. A dermal substitute that can accelerate the re-epithelialization process may greatly improve the outcome of the wound healing process. In vivo, keratinocytes rely on the basement membrane for support. Natural biological materials often retain the natural basement membrane and can therefore produce an excellent epidermis when cultured with keratinocytes.[40] A further aspect that can be assessed is the differentiation of keratinocytes into a complete stratified epidermis with all the different strata found in native epidermis when the construct is moved to the air–liquid interface. Care should be taken in selecting the source of the keratinocytes, as HaCaT cells (Spontaneously Immortalized Aneuploid Human Keratinocyte Cell Line) and foreskin keratinocytes differ from normal adult keratinocytes in their ability to produce an epidermis in vitro.[83,84]

Keratinocyte–fibroblast interactions play a vital role in directing each other's actions. When designing an in vitro experiment to assess cell–matrix interactions, one should keep in mind that results may be very different when fibroblasts and keratinocytes are co cultured on a dermal substitute compared to one cell type separately.[85] The main benefit is caused by fibroblast secreted cytokines that stimulate keratinocytes to produce a differentiated epidermis.[86,87] However it must be noted that not all fibroblast-keratinocyte interactions appear to be beneficial. The physical separation of keratinocytes and mesenchymal cells may be important in preventing keratinocytes from inducing myofibroblast differentiation.[88]

In vivo testing

Experimental methods

Animal wound models are available in various shapes and sizes.[89] Care should be taken in selecting an appropriate model for the evaluation of dermal substitutes. The first in vivo step in many dermal substitute studies is the subcutaneous implantation of a biomaterial to investigate whether the material elicits any toxicological or abnormal inflammatory responses like the foreign body response (FBR) in vivo. However the extent to which such studies may yield useful results concerning foreign body response (FBR) can be questioned. Obviously such studies do not resemble the situation in full thickness wounds, where a multitude of cellular and immunological activities are at work which can influence processes like a foreign body response (FBR) through the secretion of cytokines or cell–cell interactions not occurring in unwounded skin. Furthermore, some matrix molecules have been found to play roles in both wound healing and foreign body response (FBR) against implanted biomaterials.[90] This further suggests that the foreign body response (FBR) may be influenced by the wound healing process, which will be missed in implantation studies. Experiences with Polyactive[1] provide practical evidence for these ideas. Polyactive[1] is a synthetic material, consisting of polyethylene oxide and poly butyliterephthalate, which has been evaluated as a dermal substitute. Subcutaneous implantation studies in rats observed no discernable increase in foreign body response (FBR) to the material.[91] In a study using Polyactive[1] as a dermal substitute in Yucatan mini-pigs good dermal regeneration was reported compared to untreated wounds. A foreign body response (FBR) was reported but no detrimental effects were described.[23] However, two later studies in Gottingen mini-pig and Yorkshire pig wound models comparing Polyactive[1] to several collagen derived dermal substitutes and split-skin grafting not only found the material to result in inferior wound healing, but the Yorkshire pig study also reported a foreign body response (FBR) that caused the material to be encapsulated and even extruded from the wound bed.[92,93] This illustrates that implantation studies are not sufficient in predicting the immunological effects to implanted materials in a full thickness wound model.

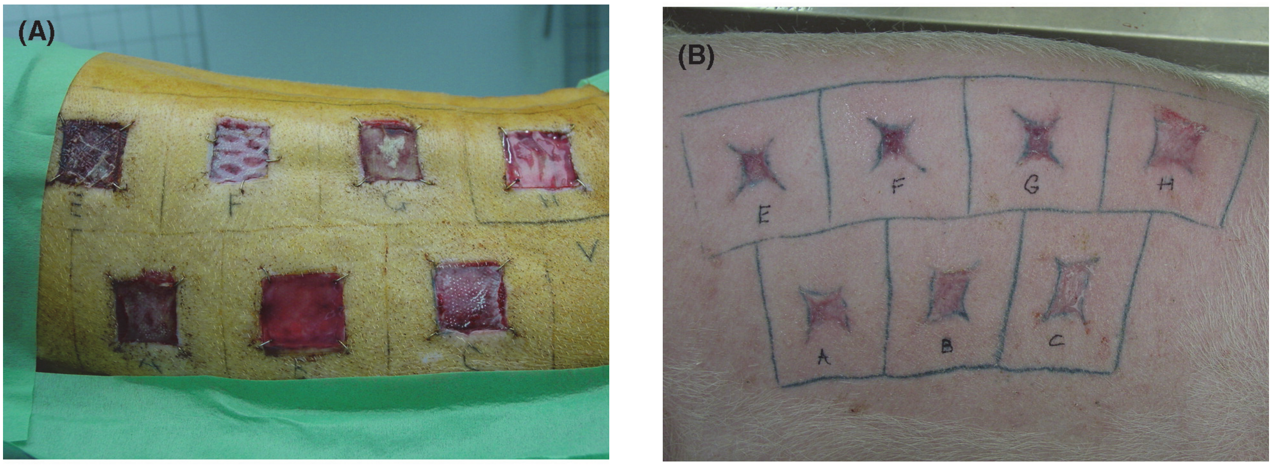
Animal species

An important factor which deserves consideration is which animal species is to be chosen for a wound model. Generally full thickness wound models have been performed in either rodents or pigs which both have their distinct advantages and disadvantages.[94] The main drawback of the use of rodents in full thickness models is their reliance on the rapid contraction of the wound to quickly close the defect, resulting in a relatively small scar compared to the original wound area. Human and porcine skin does not rely on contraction in the healing of such wounds due to differences in anatomy. Although the wound will contract, the main mechanism of wound closure is the deposition of granulation tissue which will subsequently give rise to re-epithelialization and scar formation. Studies where wound contraction in rodents is inhibited by genetic mutation[95] or mechanical restriction[96] show that loss of the contraction mechanism significantly alters many wound healing parameters in rodents. An extensive review of the literature comparing results from wound healing studies in humans, small mammals and pigs confirms that the pig is far better suited as a wound healing model for human skin than the common rodent models.[97]

Issues concerning porcine full thickness wound studies

For the reasons given above we consider the porcine full thickness wound model to be a suitable method of investigating the use of dermal substitutes in vivo (figure 5). However, the definition of a full thickness wound model is not unambiguous. Some studies use this term to refer to procedures where skin tissue is removed to the level of the subcutaneous fat[92,93] while others use it to refer to procedures completely removing all tissue up to the underlying fascia (in burn surgery this is referred to as an avulsion)[23,98,99] and yet others do not clearly specify what they mean by "full thickness".[100] A study examining repetitive removal of granulation tissue in an avulsion model demonstrated that wound contraction is not dependent on the contractile force of the granulation tissue.[98] It has been shown that subcutaneous fat tissue contains adipose derived mesenchymal stem cells[101] and that fibroblasts isolated from this tissue will readily differentiate into myofibroblasts in vitro.[102]

Figure 5. Full thickness wound model in Yorkshire pigs directly post-operative (A) and 8 weeks post-operative (B) showing marked differences in wound contraction, redness, etc.



Therefore, it could be hypothesized that the disturbance of the adipose tissue in such models could trigger an additional contractile reaction. We are not aware of any comparative studies between these two methods to date that might elucidate these observations. Investigating the differences between the two approaches might be very useful, both for reasons of basic wound healing research and the development of specific substitutes for wounds of different depths. Finally, an animal that deserves to be mentioned in relation to wound healing studies is the red duroc pig. Red durocs have been shown to develop hypertrophic scars, a problematic reaction also seen in humans but not in any of the above mentioned porcine models of wound healing.[103]

Clinical testing

The final testing system is a randomized controlled clinical trial. As shown in table 2 not all dermal substitutes have reached this final stage yet and several were only used in case reports or small scale case series, providing a low level of evidence. Very few randomized clinical trials have been performed and several considerations have to be made when interpreting the results of the clinical studies. Clinical data on dermal substitutes have been extensively reviewed elsewhere.[104–108] Therefore we will focus on relevant outcome parameters and assessments which should be taken into account when performing a clinical trial.

Table 2. Dermal substitutes

Name	Manufacturer (2009)	Materials	Thickness
Natural biological materials			
Alloderm® [44]	Lifecell Corporation, Branchburg, NJ, USA	Acellular human dermis	0.79–2.03 2.06–3.30 mm
Strattice™[a]	Lifecell Corporation, Branchburg, NJ, USA	Acellular porcine dermis (α-Gal removed)	1.5–2.0 mm
Dermamatrix	Synthes, Westchester, PA, USA	Acellular human dermis	0.2–0.4, 0.4–0.8, 0.8–1.7, 1.7> mm
Graftjacket® [136]	Wright Medical Technology, Inc, Arlington, TN, USA	Acellular human dermis	1, 1.4 or 2 mm
Oasis®	Healthpoint Ltd., Fort Worth, TX, USA	Porcine small intestine submucosa	~0.15 mm
-			-
Oasis burn matrix®			~0.30 mm
Permacol™	Covedien, Mansfield MA, USA	Acellular porcine dermis	0.4 or 1.5 mm
Tiscover®	A-SKIN B.V., Amsterdam, Netherlands	Acellular human dermis with autologous fibroblasts	1–2 mm
Glyaderm®	Euro Skin Bank, Beverwijk, Netherlands	Acellular human dermis	0.2–0.6 mm
Biological constructs			
Apligraf ® [144,145]	Organogenesis, Canton, MA, USA	Bovine collagen 1 gel with allogenic fibroblasts and keratinocytes	0.4–0.75 mm
Hyalomatrix ® [159,160]	Fidia Advanced Biopolymers, Padua, Italy	Hyaluronan based scaffold with autologous fibroblasts	~1.2 mm
Integra® (single + bilayer)	Integra Life Sciences Corp, Plainsboro, NJ, USA	Human collagen 1 with GAG	1.3 mm
Matriderm®	Skin and Health Care AG, Billerbeck, Germany	Bovine collagen 1 with elastin	1 or 2 mm
Orcel®	Forticell Bioscience New York, USA	Collagen 1 sponge + gel with allogenic fibroblasts and keratinocytes	~1 mm
Renoskin®¤	Groupe Perouse, Bornel, France	Bovine collagen 1 with GAG	1.5–2.5 mm
Synthetic materials			
Dermagraft® [171]	Advanced BioHealing, LaJolla, CA, USA	Polyglactin mesh + allogenic fibroblasts	0.19 mm
Polyactive® [174]	Octoplus Inc. (Octoplus NV Leiden)	Polyethylen oxide + polybutylitereph-thalate	~0.25 mm (250 μm)

This overview is presented as neither an all-inclusive, exclusive or an endorsement.

Pore - size	**Cross-linking**	**Application area**	**Types of clinical study**
n.a.	No	Partial and full-thickness (burn) wounds, soft tissue replacement, interpositional grafts	Non-RCT: burns [18] Case report: burns [130,131,132] Case series: acute open wounds [133,134]
n.a.	No	Soft tissue	No clinical studies available
n.a.	No	Soft tissue replacement	Comparative study: breast reconstruction [135]
n.a.	No	Chronic wounds, ligament repair, soft tissue replacement	RCT: diabetic ulcers [137,138] Case series: diabetic ulcers [139]
~20–30 µm	No	Chronic wounds	RCT: diabetic ulcers [19] RCT: chronic leg ulcer [140]
~20–30 µm	-	-	
		Partial and full-thickness burn wounds	Case report: chronic wound [142]
n.a.	Yes	Full-thickness wounds	No clinical studies available
n.a.	No	Chronic wounds	Case series: leg ulcer [143]
n.a.	No	Full-thickness wounds	No clinical studies available
n.a.	No	Partial and full-thickness (burn) wounds, skin graft donorsites, chronic wounds, Epidermolysis Bullosa	RCT: burns[144] (on top of SSG) RCT: full thickness wounds [146] RCT: chronic wounds [147,148,149] RCT: donorsites [150] Non-RCT: chronic wounds [151] Non-RCT: surgical wounds [152] Case series: full thickness wounds [153,154] Case series: epidermolysis bullosa [155,156] Case report: chronic wound [157] Case report: full thickness wound [158]
n.k.	No	Burns, chronic wounds	Case series: burns [161] Case series: burns, as temporary cover [162]
30–120 µm	Yes	Burn wounds, chronic wounds, soft tissue defects	RCT: burns [20,46,47,163] Case series: surgical wounds [164] Case series chronic wounds [165] Case report: full thickness wound [158,166]
~75 µm	No	Burn wounds, chronic wounds	RCT: burns [21] Comparative study: burns [167] Case series: full thickness wounds [48,168]
50–250 µm	Yes	Chronic wounds skin graft donorsites,	RCT: donorsites [169] Case series: reconstruction wounds [170]
~100 µm	Yes	Burn wounds, tissue defects	No clinical studies available
280 × 400 µm	No	Burn wounds, chronic wounds, diabetic ulcers	RCT: chronic wounds [172,173] Comparative study: burns [22]
100–200 µm	No	Bone, cartilage repair	No clinical studies available

Products may be currently off the market. n.a.: Not applicable; n.k.: Not known. ¤ Including temporary silicone layer.

Assessment

In clinical studies, the most common short-term outcome parameters are graft take of the autograft and time to wound healing. Graft take and time to wound healing can be influenced by many factors. Among these factors are wound bed preparation, control of microbial contamination, location of the wound, dressing care, and survival of the transplanted cells during vascularization of grafts.[109] These outcomes are usually measured subjectively by the clinician since no objective tools are readily available for this purpose.

Long-term outcome parameters are functional and cosmetic quality of the scars.

The assessment of these outcome parameters should ideally consist of a subjective and objective scar assessment scale and should include a sufficient follow-up time.[110,111] Various scar assessment scales and measurement tools are available today [112] but the most commonly used scale is the Vancouver Scar Scale (VSS).[113] Drawbacks of this subjective evaluation are that not all items are present in equal weight, e.g. hypopigmentation is judged as a lighter condition in terms of scarring than hyperpigmentation. Other scales were designed to overcome these difficulties.[114–116] The Patient and Observer Scar Assessment Scale (POSAS) contains the most important scar characteristics and includes a patient's opinion. All parameters are scored on a 10-step scale enabling the physician to produce a mean scar score.

Several non-invasive instruments are available to objectively determine the different scar features. Table 3 shows examples of currently commercially available tools.

The actual use of such instruments in clinical trials is still scarce, but should be recommended in order to improve the level of evidence for effectiveness of dermal substitutes.

Application of dermal substitutes

Table 2 gives an overview of currently commercially available dermal substitutes according to the classification used in this review. Only permanent dermal substitutes were included, because we considered temporary substitutes as wound dressings. Substitutes that explicitly act as an epidermal cover, were also excluded.

Table 3. Currently commercially available tools

Scar features	Objective instrument
Color (consisting of redness and pigmentation)	DermaSpectrometer [117,118], Mexameter [119,120], Chromameter [117]
Thickness	TUPS[a] [121,122], Dermascan C [119,120]
Surface roughness	PRIMOS[b] [123], Transparency- and Laser profilometry [124,125]
Pliability/elasticity	Cutometer [126,127], Dermal torque meter [128]
Surface area (Planimetry)	Visitre [129]

Conclusion and summary

Dermal substitutes are of major importance in treating full thickness skin defects, both in acute and chronic wounds. We outlined specific requirements of three classes of dermal substitutes:

- natural biological materials, with a more or less intact extracellular matrix structure;
- constructed biological materials, composed of specific biological components; and
- synthetic substitutes, which can be synthesized on demand and can be modulated for specific purposes.

Biological and clinical requirements were translated to composition, physical structure, immunological properties and cell–matrix interactions of the various materials.

Important substitute properties like pore size, cell adhesion sites (e.g. RGD sequences), crosslinking, degradability and the presence of a basement membrane were discussed for each of the different classes of materials.

Each class had specific properties that made it either suitable for or caused problems with specific applications. The natural materials offer cells a native extracellular matrix (ECM) which may allow them to construct more natural new dermis. They also provide excellent re-epithelialization characteristics due to the presence of the basement membrane, but exhibit problems with vascularization in a one-step procedure due to slow vascularization of the material.

Constructed materials demonstrate the advantages of increased control over scaffold composition, as the addition of extracellular matrix components to the material allows products to influence the behavior of the invading cells. This results in materials such as Matriderm1 that overcame the problems with vascularization seen in natural materials and earlier constructed materials like Integra1. Scaffold crosslinking may result in cytotoxicity or foreign body responses, which still need to be investigated further in order to avoid such problems.

Synthetic materials have not yet reached the stage where they can be clinically applied as dermal substitutes, but research is promising and many problems associated with these types of materials have been overcome in vivo. Incorporation of biomimetic proteins that provide cells with binding and degradation sites may solve the problems with biocompatibility of synthetic materials observed in full thickness wound models.

Furthermore, we discussed various stages of dermal substitute testing. In vitro test systems are limited in their ability to mimic all physiologically relevant properties of skin. Nevertheless, cytotoxicity, cell proliferation and migration and even cell differentiation and function can easily be tested using in vitro cell culture and tissue wound models. For in vivo testing of dermal substitutes relevant animal models are available and should be chosen in accordance with the research questions. For in vivo dermal substitute testing, porcine full thickness wound models should be the experimental model of choice. These procedures replicate most closely the conditions which dermal substitutes are designed to heal; large wounds that do not heal by primary closure and involve a substantial loss of dermis, for instance a burn wound after debridement. The use of pigs is recommended as skin healing in rodents and humans differs too much in important aspects. Randomized clinical trial data on effectiveness of dermal substitutes are not available for all substitutes and clinical evidence often consists of case reports.

We advocate more comparative studies of different substitutes in trial designs using measurable outcome parameters. We think this will provide a new impetus for dermal substitutes towards the ultimate goal of effective and scar free wound healing.

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Chapter 3

Dermal substitution in acute burns and reconstructive surgery: 12-Year Follow-up

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Abstract

Background

Application of dermal substitutes has been reported to improve the outcome of burns. However, the long-term effectiveness of dermal substitutes has not been investigated objectively. The aim of this study was to evaluate long-term effectiveness of a collagen-elastin dermal substitute in acute and reconstructive burn surgery.

Methods

From 1996 to 1998, an intra-individual comparison was carried out between a dermal substitute with a split-skin graft and a split-skin graft alone in patients with acute and reconstructive wounds. In this follow-up, scar elasticity, vascularization, pigmentation, and surface roughness were determined objectively. In addition, a subjective scar assessment was performed.

Results

In 46 patients, 69 pairs of substituted and conventionally treated sites were measured, consisting of acute and reconstructive burn scars. In reconstructive scars, one surface roughness parameter was significantly better in substituted scars. Subjective assessment in acute and reconstructive burn scars showed several statistically significant differences in favour of substituted scars, such as pliability, relief, and the general observer score. Elasticity measurements showed higher scores for substituted scars, although the difference was not statistically significant. For the subcategory of scars treated with a largely expanded meshed skin graft, a significantly higher elasticity was found for the substituted area.

Conclusion

In this first long-term and objective follow-up of dermal substitution, the authors found improved scar parameters in both acute and reconstructive wounds treated with the substitute, indicating a long-lasting effect on scar quality.

Introduction

In recent years, the aim of wound healing has been rapid wound closure; also, the functional and cosmetic outcome of scars has become increasingly important. To improve scar outcome, several dermal substitutes have been developed. The use of dermal substitutes in acute and reconstructive wounds is thought to lead to better scar function (e.g., elasticity and mechanical stability) and scar appearance. This could be attributable to the fact that a dermal substitute serves as a support structure for the ingrowth of vessels and autologous fibroblasts.[1–4] In recent years, multiple studies have shown good results with the use of dermal substitution in acute and reconstructive wounds.[4–12] However, there is a paucity of studies with objective evaluation and long-term follow-up. Consequently, little information is available on long-term effectiveness of dermal substitutes. This is important to assess functional and cosmetic scar outcome; also, the need for reconstructive surgery and quality of life can be established.

Previously, our group reported the results of this trial and stated several important conclusions. First, the successful application of a dermal substitute in a one-stage procedure was shown, which implies that only one operation is needed and the risk of infection is reduced.[13] Second, a statistically significant increase of elasticity was seen in reconstructive wounds treated with the substitute, compared with wounds treated with a split-skin graft alone, at 3 months postoperatively.[13] Twelve months postoperatively, the absolute difference in elasticity between substituted and reference scars was the same (0.04 mm). However, elasticity had improved in both scars and thus this difference was not significant. In a separate analysis of acute burn scars, elasticity was higher in substituted scars treated with a large expansion graft.[14] Furthermore, in the follow-up 12 months postoperatively, substituted scars seemed smoother compared with reference scars, especially in combination with a largely expanded graft.[14] At that time, no tool was available to measure and objectify scar surface roughness. In recent years, several additional objective tools have been developed, including a tool that measures surface roughness and a tool that quantifies pigmentation and vascularization.[15,16] Also, an improved subjective assessment scale was developed (Patient and Observer Scar Assessment Scale). Therefore, several scar aspects can be investigated subjectively and objectively at present. This article presents the first subjective and objective results of a 12-year follow-up of dermal substitution in acute and reconstructive burn wounds.

Patients and methods

Between 1996 and 1997, 62 burn patients were included in a clinical controlled trial of the use of a collagen-elastin matrix as a dermal substitute.[13,14,17]

Patients were eligible if they were admitted to our hospital and needed surgical treatment for acute burn wounds or reconstruction of burn scars. In all patients who gave written informed consent, a paired intra-individual comparison was made: one part of the wound was treated with the conventional split-skin graft and the other part of the wound was treated with the dermal substitute and a split-skin graft. These areas were anatomically related areas in which a right/left, superior/inferior, or medial/lateral comparison was made. Presently, all patients were addressed for follow-up by means of a letter with an explanation of the assessment. After confirmation of their participation, an appointment was made in the Red Cross Hospital, Beverwijk, The Netherlands.

Study design

In each patient, the experimental scar (treated with the dermal substitute and a split-skin graft) and the reference scar (treated with a split-skin graft alone) were located. With the use of a well-documented photographic archive and precise wound descriptions, scar sites were found 12 years postoperatively (figures 1 and 2). Then, scars were examined by four methods of scar evaluation. The Cutometer, the DermaSpectrometer, the Phaseshift Rapid In Vivo Measurement of Skin, and the Patient and Observer Scar Assessment Scale were used to obtain objective and subjective information of scar characteristics and are described separately below.

Scar elasticity

The Cutometer Skin Elasticity Meter 575 (Courage & Khazaka GmbH, Cologne, Germany) is a reliable and valid instrument for evaluation of skin elasticity.[18] It measures the vertical deformation of skin in millimeters during a controlled vacuum. In all patients, the measuring probe was placed in the scar center. The following elasticity parameters are provided by the Cutometer: maximal skin extension, pliability, elasticity, retraction, and viscoelasticity.

Scar erythema and melanin

The DermaSpectrometer (Cortex Technology, Hadsund, Denmark) is a validated assessment tool that emits light by means of diodes at two defined wavelengths: green light (568 nm) and red light (655 nm).[19] Photodetectors measure the light reflected by the skin.

Figure 1. Clinical application of the dermal substitute for burns. The substitute was applied on the right side of the chest (above, left). Dermal substitute is on the reader's left; control is on the right. Both sides were covered with a meshed split-skin autograft (1:3) (above, right). Dermal substitute and autograft are on the reader's left; control (autograft only) is on the right. The appearance of the scar of the substituted area seemed smoother 10 months postoperatively (below, left).[14] Scar appearance 11.6 years postoperatively is presented (below, right).

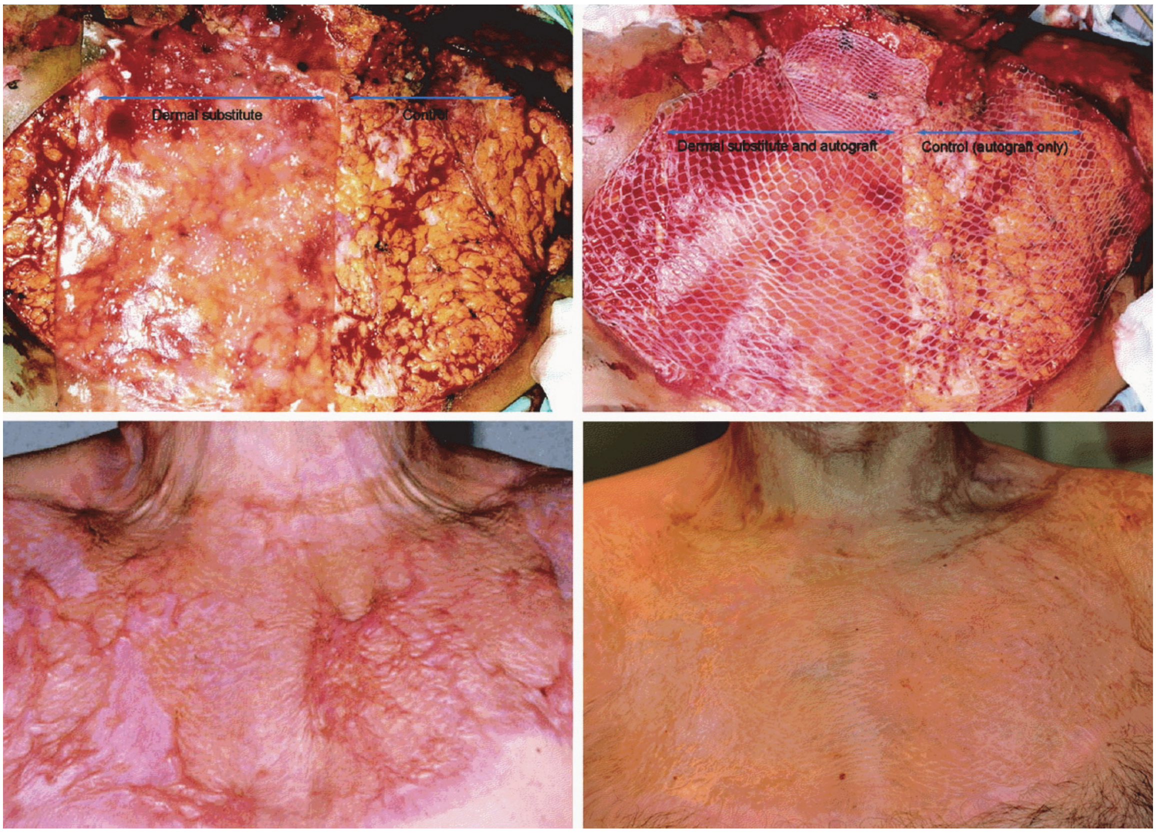
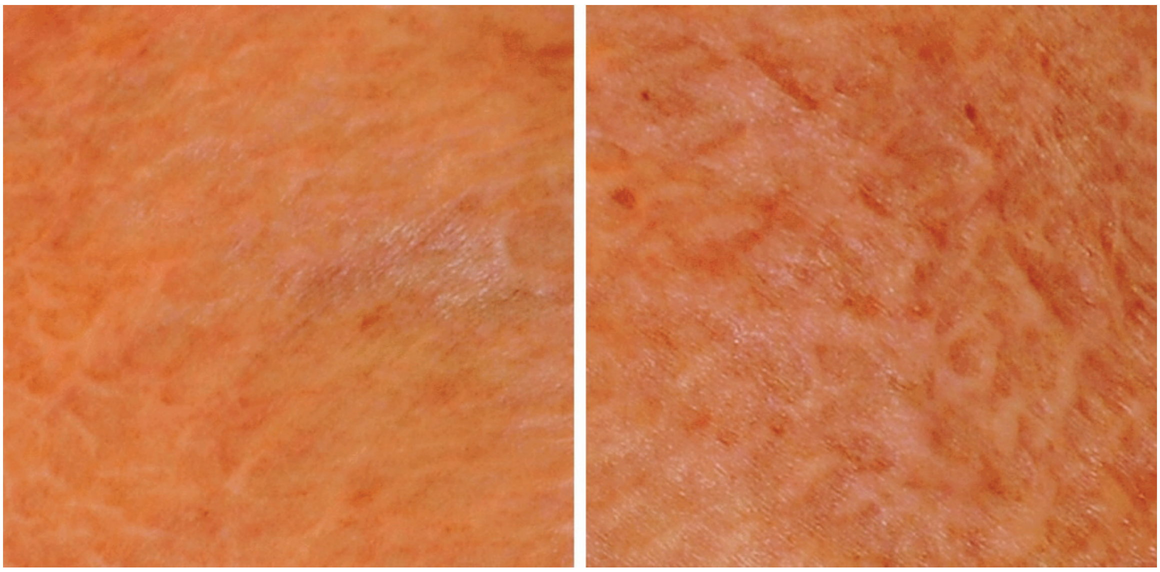


Figure. 2. (Left) Close-up of substituted scar. (Right) Close-up of reference scar.



As green light is absorbed by hemoglobin and red light is absorbed by melanin, an erythema and melanin index can be computed, based on the intensity of absorbed and reflected light. The 6-mm-diameter probe was placed gently in the scar center.

Scar surface roughness

The Phaseshift Rapid In Vivo Measurement of Skin (GFMesstechnik GmbH, Berlin, Germany) was used for evaluation of surface roughness. This measuring device produces a three-dimensional image of the microtopography of the skin.[20,21] The system works with a digital stripe projection technique, which is projected onto the skin. Accordingly, a three-dimensional image is achieved by elevation differences on the measured area. Calculation of scar surface roughness was carried out within an area measuring 39 x 29 mm, using Phaseshift Rapid In Vivo Measurement of Skin software version 5.6. The measuring frame was placed in the scar center. When the scar area was smaller than the frame, only the exact study area was selected for evaluation. The following surface roughness parameters were used to determine scar roughness: the arithmetic mean of the surface roughness (measured in micrometers) (Sa), the mean of the five highest peaks and five deepest valleys from the measuring field (measured in millimeters) (Sz), and the number of peaks per unit length (peak count, or PC). Figures 3 and 4 and table 1 demonstrate the use of the Phaseshift Rapid In Vivo Measurement of Skin in one of the patients.

Table 1. Phaseshift Rapid In Vivo Measurement of Skin: data of study patient shown in figures 3 and 4

	Substitute	Reference	Normal skin
Sa, µm	29.5	47.5	25.0
Sz, mm	350.0	579.0	288.0
PC	24.5	61.5	9.0

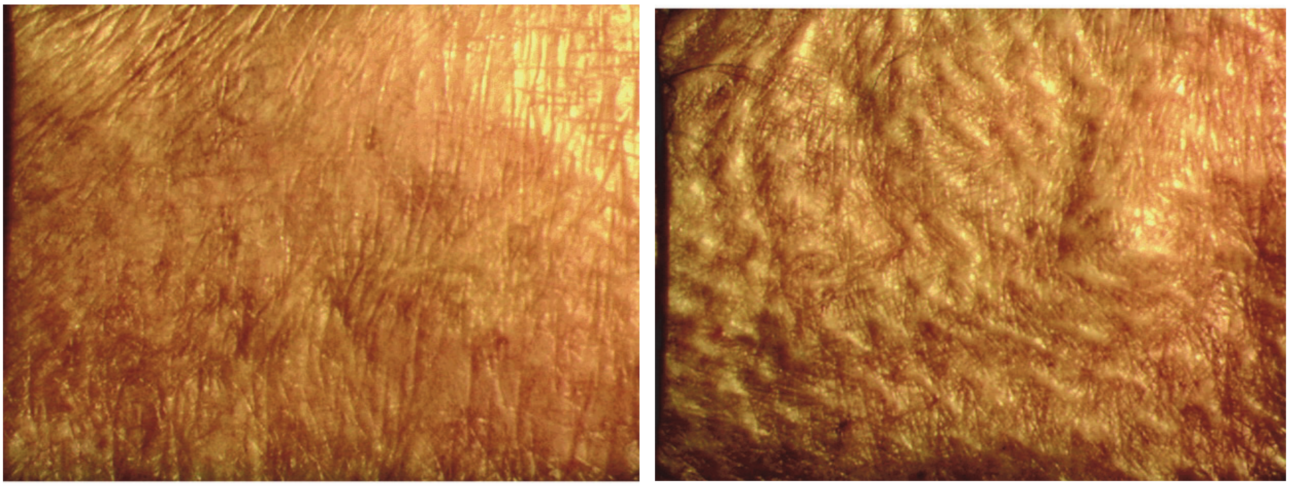
Sa: arithmetic mean of the surface roughness, Sz: mean of the five highest peaks and five deepest valleys from the measuring field, PC: peak count (number of peaks) of substituted scar, reference scar, and normal skin.

Subjective scar evaluation

The Patient and Observer Scar Assessment Scale is a validated scar assessment tool that consists of two scales, the patient scale and the observer scale.[22,23] The patient scores scar characteristics colour, pliability, thickness, relief, itching, and pain. The observer scale contains the items vascularization, pliability, pigmentation, thickness, relief, and surface area. All items are scored numerically by using a 10-step score, in which a score of 10 reflects the worst imaginable scar or sensation. The total observer score and the total patient score consist of adding the scores of the six items (range, 6 to 60). The lowest total score reflects normal skin. In addition to these items, the observer and the patient give a general opinion of the appearance of the scar (score, 1 to 10, in which a score of 10 corresponds to the worst possible scar). In each patient, the observer assessment was performed by three experienced researchers.

Figure. 3. Use of the Phaseshift Rapid In Vivo Measurement of Skin in a study patient. Photograph of the right hand (substituted scar) and the left hand.

Figure 4. Use of the Phaseshift Rapid In Vivo Measurement of Skin, continued. (*left*) Live picture of a hand treated with substitute and split-skin graft (substitute) obtained by means of the Phaseshift Rapid In Vivo Measurement of Skin. (*right*) Live picture of a hand treated with a split-skin graft alone (reference) obtained by means of the Phaseshift Rapid In Vivo Measurement of Skin.



Materials

Experimental wounds were treated with a split-skin graft and the precursor of the dermal substitute, Matriderm (Dr. Otto Suwelack Skin & Health Care AG, Billerbeck, Germany). This three-dimensional matrix is a highly porous membrane composed of a native bovine type I, III, and V collagen fiber template. The collagen fibers are coated with α-elastin hydrolysate derived from the bovine nuchal ligament in a concentration of 3% weight-to-weight ratio (GfN-Herstellung von Naturextracten GmbH, Strassburg, Germany). The substitute was treated with γ-irradiation (approximately 1000 Gy) and stored at room temperature. In this study, matrices with 1-mm thickness were applied in a single-stage grafting procedure.

Statistical analysis

Data were analyzed by using SPSS for Windows version 16.0 (SPSS, Inc., Chicago, Ill.). After being tested for normality, a paired t test was applied. Non-normally distributed data were analyzed by means of the Wilcoxon signed rank test. The significance criterion for all tests was set at 0.05.

Results

In total, 46 of the initial 62 patients participated in this follow-up, consisting of patients with acute and reconstructive burn wounds. Six patients had participated in the acute burn group, after which they were included in the reconstructive group, with other wound sites. A responsiveness of 79% was therefore reached for this follow-up (figure 5). Table 2 demonstrates several patient characteristics of the study group.

Figure 5. Patient flow chart.

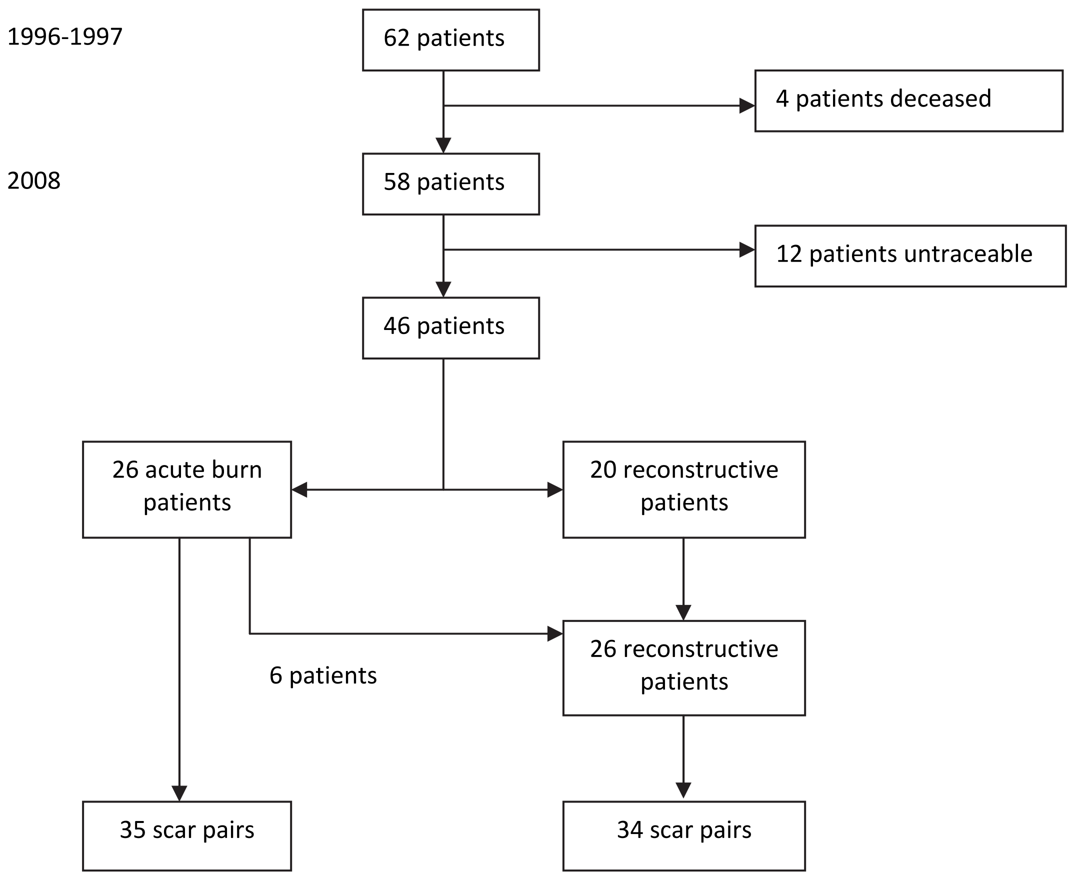


Table 2. Patient characteristics

Characteristics	Value
Acute	
No. of patients	26
No. of scar pairs	35
Male sex	15 (58%)
Mean TBSA burned, %	24.3 ± 14.7
Mean full thickness, %	11.8 ± 14.1
Mean age at follow-up, yr	41.3 ± 18.7
Mean length of follow-up period, yr	11.8 ± 0.4
Reconstructive	
No. of patients	26
No. of scar pairs	34
Male sex	16 (62%)
Mean TBSA burned, %	30.5 ± 17.6
Mean full thickness, %	20.9 ± 15.0
Mean age at follow-up, yr	42.3 ± 18.2
Mean length of follow-up, yr	11.8 ± 2.1

TBSA: total body surface area.

Elasticity

In the acute burn group, no differences were seen between substituted and reference scars. In the reconstructive scars, all elasticity parameters were higher in substituted scars, although the difference was not statistically significant (table 3). Table 3 also shows the improvement of elasticity compared with previous results at 3 and 12 months postoperatively, which are presented in table 4.

Table 3. Results of elasticity measurements 12 years postoperatively

	Substitute at 12 Years[a]	Reference at 12 Years[b]	p (CI 95%)	Improvement compared with 12-month results (t Test) (%)	
Acute, mm					
Extension	0.40	0.40	0.991 (–0.05 to 0.05)	82	82
Pliability	0.26	0.28	0.244 (–0.06 to 0.02)	53	56
Elasticity	0.33	0.31	0.405 (–0.02 to 0.06)	120	94
Retraction	0.22	0.23	0.393 (–0.05 to 0.02)	57	53
Viscoelasticity	0.07	0.09	0.134 (–0.04 to 0.01)	0	50
Reconstructive, mm					
Extension	0.46	0.43	0.368 (–0.04 to 0.10)	15	26
Pliability	0.34	0.32	0.502 (–0.03 to 0.07)	-3	7
Elasticity	0.37	0.34	0.389 (–0.03 to 0.08)	32	42
Retraction	0.30	0.29	0.521 (–0.03 to 0.06)	3	26
Viscoelasticity	0.09	0.08	0.365 (–0.01 to 0.02)	-25	-20

CI: confidence interval.

[a] Treated with the dermal substitute and a split-skin graft.

[b] Treated with a split-skin graft alone (control treatment).

Table 4. Results of elasticity measurements 3 and12 months postoperatively [13,14]

	3 months			12 months		
	Substitute[a]	**Reference**[b]	**p (t Test)**	**Substitute**[a]	**Reference**[b]	**p (t Test)**
Acute, mm						
Extension	0.18	0.18	NS	0.22	0.22	NS
Pliability	0.14	0.14	NS	0.17	0.18	NS
Elasticity	0.13	0.13	NS	0.15	0.16	NS
Retraction	0.12	0.12	NS	0.14	0.15	NS
Viscoelasticity	0.05	0.05	NS	0.07	0.06	NS
Reconstructive, mm						
Extension	0.24	0.18	0.002*	0.40	0.34	NS
Pliability	0.21	0.14	0.001*	0.35	0.30	NS
Elasticity	0.16	0.12	0.041*	0.28	0.24	NS
Retraction	0.17	0.13	0.011*	0.29	0.23S	NS
Viscoelasticity	0.08	0.06	0.079*	0.12	0.10	NS

CI: confidence interval, NS: not significant.
[a] Wound sites treated with the dermal substitute and a split-skin graft.
[b] Wound sites treated with a split-skin graft alone (control treatment).
* <0.05.

Erythema and melanin

No statistically significant differences in erythema and melanin were found between substituted and reference scars in the acute burn group (data not shown). In reconstructive scars, a statistically significant difference was found between melanin of substituted and reference scars. Melanin differed more from patient's normal skin in substituted scars compared with reference scars, although the difference was small (5%) (substitute-to-normal skin ratio, 1.05, reference-to-normal skin ratio, 1.00; p < 0.010).

Table 5. Results of surface roughness measurements

	Substitute[a]	**Reference**[b]	***p* (Wilcoxon Signed Rank Test)**	**Normal skin**
Acute				
Sa	32.6	36.4	0.061	19.6
Sz	417.6	448.5	0.604	291.8
PC	25.3	34.1	0.083	16.3
Reconstructive				
Sa	53.9	58.7	0.275	23.1
Sz	709.7	931.7	0.014‡	303.6
PC	41.2	52.0	0.172	14.0

CI: confidence interval, Sa: arithmetic mean of the surface roughness (mm), Sz: mean of five highest peaks and five deepest valleys from the measuring field (mm), PC: peak count, number of peaks.
[a] Treated with the dermal substitute and a split-skin graft.
[b] Treated with a split-skin graft alone (control treatment).
* $p < 0.05$.

Surface roughness

In the acute burn group, no statistically significant difference between substituted and reference scars was seen in surface roughness. However, the three roughness parameters were lower (better) in substituted scars. In the reconstructive group, all three parameters showed lower scores in substituted scars compared with conventionally treated areas, of which the mean of the five highest peaks and five deepest valleys from the measuring field differed statistically significantly (table 5).

Subjective evaluation

Subjective scar assessment in the acute burn group showed a statistically significant difference in favour of the substituted scars in all items, except for vascularization (figure 6). In the reconstructive group, observer scores for pliability, relief, and the general score also showed significantly lower (better) results for substituted scars (figure 7). Patient scores did not show significant differences in either groups (data not shown), except for scar relief in the acute group (substitute, 3.2; reference, 4.0; p = 0.034).

Figure 6. Subjective evaluation of acute burn scars. Error bars represent ±2 SE. Substitute wounds are those wound sites treated with the dermal substitute and a split-skin graft (blue). Reference wounds are those wound sites treated with a split-skin graft alone (control treatment) (green). POSAS, Patient and Observer Scar Assessment Scale (*p < 0.02).

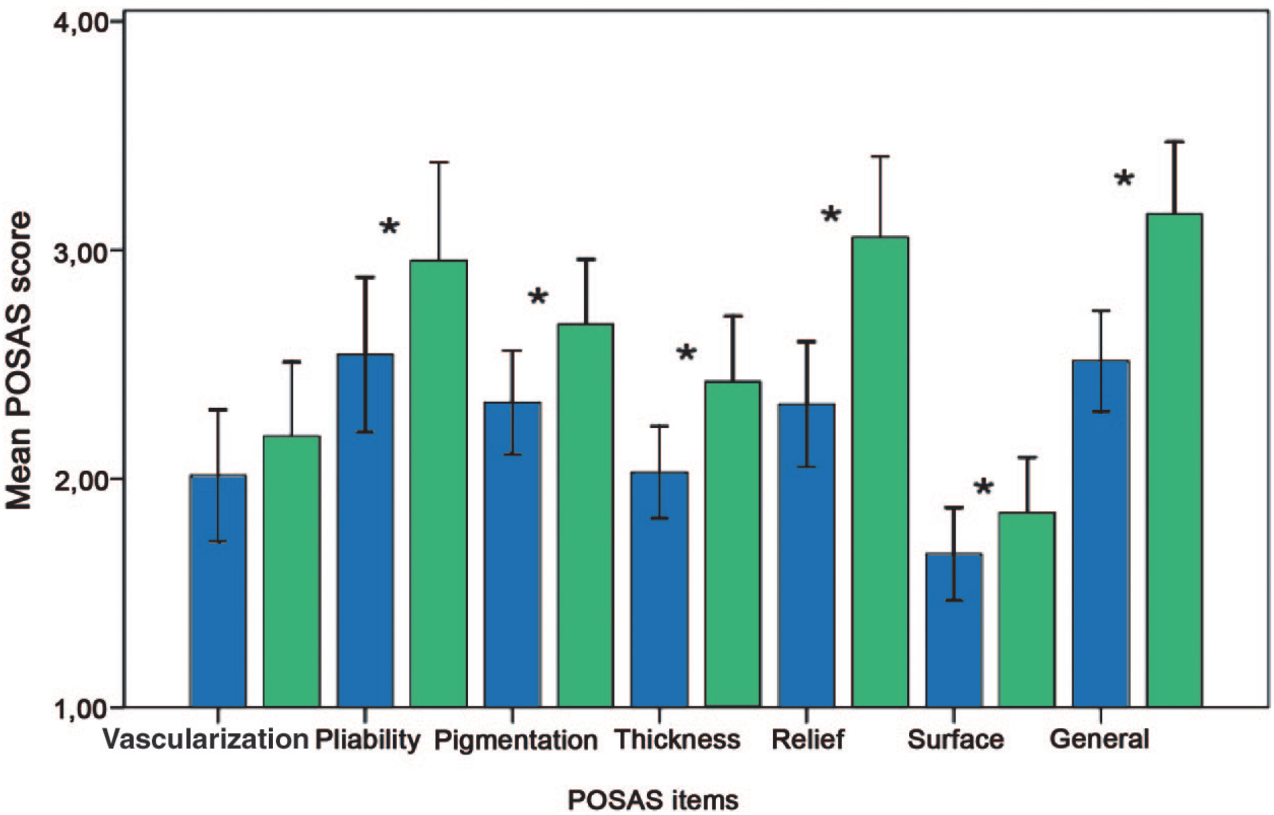
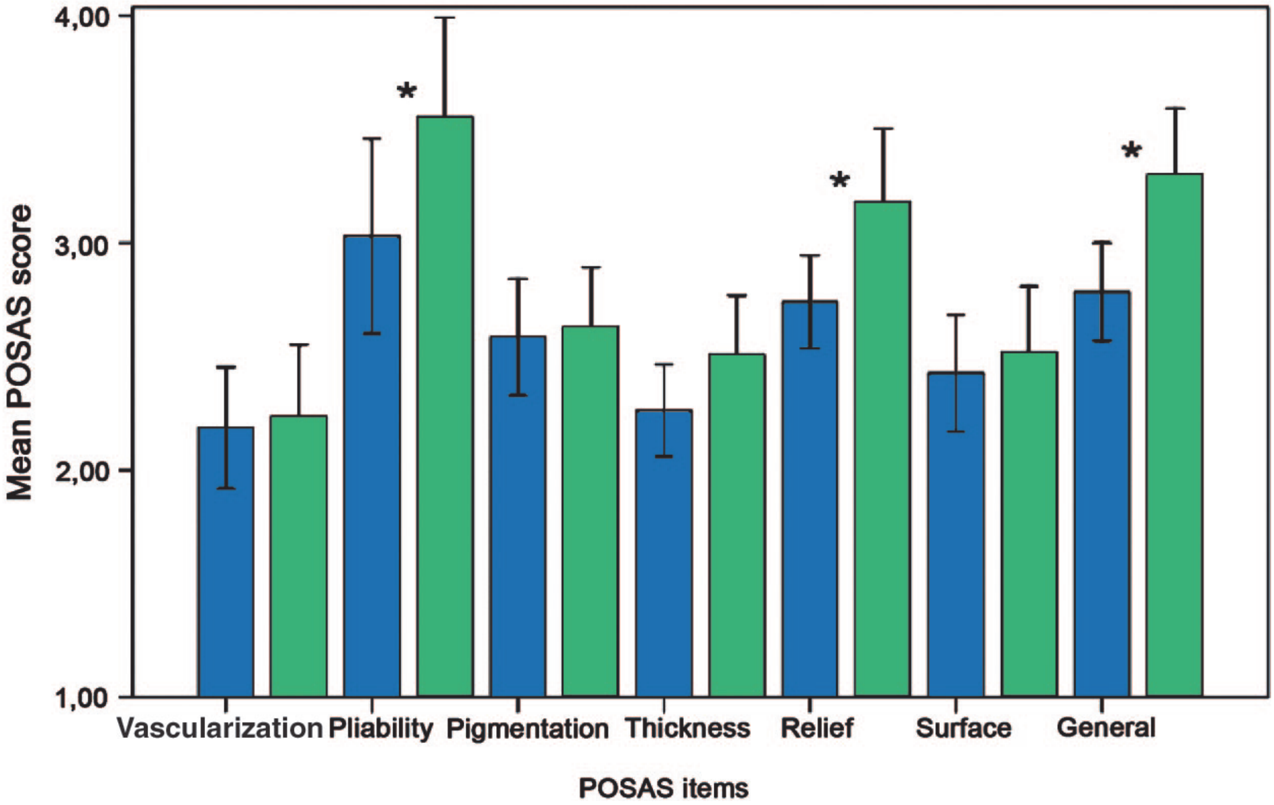


Figure. 7. Subjective evaluation of reconstructive burn scars. Error bars represent ±2 SE. Substitute wounds are those wound sites treated with the dermal substitute and a split-skin graft (blue). Reference wounds are those wound sites treated with a split-skin graft alone (control treatment) (green). POSAS, Patient and Observer Scar Assessment Scale (*p < 0.04)



Substitution and graft expansion

In the treatment of acute burn wounds, different mesh expansions were used for the split-skin grafts. Small expansions (mesh, 1:1.5 and 1:2) were used for 20 wound pairs, and larger expansions (mesh, 1:3 and 1:4) were applied in 15 pairs. A separate analysis was performed in this group, as we found interesting differences 12 months post-operatively. At present, significant results were seen for the elasticity parameters pliability, retraction, and viscoelasticity in the small expansion group. In these parameters, substituted scars showed lower elasticity scores than reference scars. In the group with larger expansions, significant differences for the elasticity parameters maximal skin extension, elasticity, and retraction were seen. Substituted scars showed higher scores of elasticity than reference scars (table 6). Measurements of vascularization, pigmentation, and surface roughness showed no statistically significant differences between substituted and reference scars. Nevertheless, in both expansion groups, the majority of the roughness parameters were lower (better) in substituted scars (data not shown). The subjective evaluation in the small expansion group showed significantly lower (better) scores for relief, surface area, and the total score in substituted scars. In the larger expansion group, all items received significantly lower scores in substituted scars, except for the item surface area (data not shown).

Table 6. Elasticity in acute burn scars with small and large graft expansions

Elasticity parameters	Small expansion			Large expansion		p (Wilcoxon Signed Rank Test)
	Substitute[a]	Reference[b]	p	Substitute[a]	Reference[b]	
Extension	0.39	0.46	0.073	0.42	0.32	0.008*
Pliability	0.24	0.31	0.003*	0.28	0.24	0.056
Elasticity	0.33	0.36	0.332	0.34	0.26	0.009*
Retraction	0.21	0.26	0.004*	0.24	0.20	0.026*
Viscoelasticity	0.07	0.11	0.000*	0.08	0.07	0.100

[a] Wound sites treated with the dermal substitute and a split-skin graft.
[b] Wound sites treated with a split-skin graft alone (control treatment).
* p < 0.05

Discussion

This study presents the first 12-year follow-up with subjective and objective evaluation of dermal substitution. The clinical effectiveness of a collagen-elastin matrix was evaluated in 46 patients, 12 years after application.

For the first time, an objective evaluation tool for surface roughness was used to investigate scar outcome of dermal substitutes. At our previous assessment, a smoother surface was seen in substituted scars; therefore, we anticipated detecting differences between substituted and reference scars.[14] In addition, this scar aspect is important to measure, because an irregular scar surface can be cosmetically disturbing and long-lasting, despite scar maturation. In reconstructive scars, the mean of the five highest peaks and five deepest valleys from the measuring field was significantly lower in substituted scars, which implies a smoother surface of these scars (table 5). These results support the clinical observation, as numerous substituted scars in this study showed a reduced visibility of the mesh pattern (figures 1 and 2). A hypothesis is that the hypertrophy that appears in the interstices of the autograft is less when a dermal substitute is used. The dermal substitute seems to replace the dermis and bridge the interstices of the autograft. This may explain the smoother aspect of the scar.

In an earlier evaluation of the reconstructive group, elasticity was higher in substituted scars compared with reference scars, although no statistically significant difference was found (table 4).[14] For this reason, no significant difference in elasticity was expected at present. In this follow-up, a comparable difference in elasticity between substituted and reference areas was found. Similar to the results at 12 months postoperatively, scores were higher in the reconstructive scars treated with the substitute compared with reference scars, with no significant differences. Apparently, the presence of the substitute in the early phase of wound healing contributed to a higher elasticity, even after 12 years. It has to be taken into consideration that a different Cutometer was used in our previous evaluations (i.e., Cutometer 474). Our results are in line with other published studies and patient series. An improvement of elasticity (evaluated in a subjective manner) was found in scars treated with Matriderm or Integra (Integra LifeSciences Corp., Plainsboro, N.J.).[9,24–26]

In our previous report on the results at 12 months postoperatively, the effectiveness of the dermal substitute seemed to be related to the expansion rate of the overlying mesh graft.[14] Because this was an interesting finding, a separate analysis of the acute burn group was repeated to compare the effect of the substitute with small and larger mesh expansions. A higher elasticity was anticipated in scars treated with a smaller expansion and the elasticity of the reference scars in both groups seemed to confirm this hypothesis. However, elasticity of substituted scars was almost the same with both large and small expansions (Table 6). In addition, elasticity of substituted scars with a larger expansion graft was higher than that of non-substituted scars with this expansion. In contrast, in substituted scars treated with small graft expansions, elasticity was lower than in reference scars.

These findings suggest that dermal substitution contributes to a higher elasticity, mainly in combination with a larger expansion. To explain the lower elasticity in substituted scars treated with a small expansion graft, the following hypothesis is described. The measured elasticity in substituted scars with a small expansion may be lower because of the presence of a thicker dermis at this particular point in time, up to 12 years after surgery. For normal skin, a thicker dermis leads to a lower elasticity as measured by the Cutometer. For example, skin elasticity of an arm is lower than skin elasticity of the hand dorsum because of a thicker dermis. It must be noted that this hypothesis is contradictory, as results of the Cutometer are usually interpreted the opposite way (i.e., a higher score of elasticity is a better scar feature). We assume, however, that this is true for short follow-up periods up to 1 year after transplantation. In more mature scars, as measured in this study, the presence of more dermis may lead to opposite results. For now, the clinical relevance of this finding is that dermal substitution is especially beneficial in combination with a largely expanded autograft.

Subjective observer scar assessment at 12 months postoperatively, using the Vancouver Scar Scale, showed no significant differences between substituted and reference scars. At present, the Patient and Observer Scar Assessment Scale was used, as this scale has been reported to be more reliable and valid in the assessment of burn scars.[22]

Considering our previous results, a large difference between substituted and reference scars was not expected. However, subjective assessment in both groups showed significantly lower scores for substituted scars in several scar items (figures 6 and 7). Thus, although differences between substituted and reference scars were subtle, the appearance of substituted scars was found to be better. However, it should be noted that the observers of this study could not be blinded; therefore, a bias cannot be excluded. It was remarkable that subjective scores were relatively low for all scars. This is probably attributable to prolonged scar maturation, as previously suggested in the prior report of this study.[14] Our subjective results are comparable to the results of other clinical studies on the use of dermal substitutes.[5,9,14,24,25]

Before discussing the limitations of this follow-up, we will first consider the limitations of the original study. At that time, no randomization procedure was used for choosing the treatment of the wound pair. The matrix was always applied on the right, superior, or medial side of the wound. In addition, this procedure made a blind study design during scar evaluation impossible. Furthermore, in patients with one wound only, two different therapies were used within the same wound. It is possible that treatments affected each other.

In this follow-up, a few limitations arose as well. First, some study areas were difficult to trace after 12 years. Because of improvement of these study areas, distinguishing the substituted scar from the reference scar or even the surrounding skin or scar was complicated, especially when repeated corrective procedures were applied in that area. Original study areas that could not be retraced or measured were excluded from the follow-up. A second limitation of this follow-up was the subjective evaluation of patients. It was difficult for patients with a high percentage total body surface area burned to give their opinion on a relatively small scar. Nevertheless, the total patient score of substituted scars was lower compared with the total score of reference scars (substitute, 20.1; reference, 21.6; p=0.139). Despite these limitations, 69 scar pairs were suitable for long-term evaluation.

Conclusions

This article presents the first study that objectifies scar outcome after 12 years with the use of a collagen-elastin matrix in acute and reconstructive burn surgery. Even after this long period, we found improved scar parameters in both acute and reconstructive substituted wounds. As scar surface roughness was objectified, we found that substituted areas were significantly smoother than areas treated with a split-skin graft only. Another important finding was the increased elasticity in substituted scars treated with a largely expanded autograft. The results of this follow-up indicate a long-lasting effect of dermal substitution on scar quality. Further research in particular, randomized controlled trials is needed to obtain more data on the effectiveness of dermal substitutes.

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Part II
Intralesional cryotherapy for treatment of keloid scars

Chapter 4

Intralesional cryotherapy for the treatment of keloid scars: evaluating effectiveness

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Abstract

Introduction

Intralesional (IL) cryotherapy is a novel treatment technique for keloid scars, in which the scar is frozen from inside. Over the past decade, several studies have been published with varying outcomes. A critical analysis of the current literature is therefore warranted to determine whether IL cryotherapy is an alternative to established keloid scar treatments.

Method

A comprehensive review was performed, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). PubMed and Embase were searched from inception. Studies and level of recommendation were graded according to the American Society of Plastic Surgeons criteria.

Results

Eight studies meeting the inclusion criteria were selected. The average volume decrease ranged from 51% to 63%, but no complete scar eradication was achieved on average. Scar recurrence ranged from 0% to 24%. Hypopigmentation post-treatment was seen mostly in Fitzpatrick 4-6 skin type patients. Finally, complaints of pain and pruritus decreased significantly in most studies.

Conclusion

IL cryotherapy for the treatment of keloid scars shows favorable results in terms of volume reduction and alleviated complaints of pain and pruritus. However, no complete scar eradication is established and recurrences are seen. Also, persistent hypopigmentation proved a problem in Fitzpatrick 4-6 skin type patients. Summarized, the evidence proved limited and inconsistent resulting in an ASPS grade C recommendation for this type of treatment of keloid scars.

Introduction

In predisposed individuals, injury of the skin can lead to an abnormal healing response resulting in keloid scars.[1] Besides aesthetic disfigurement, keloids can cause major physical complaints of pain and pruritus, hence impairing the quality of life of the patient.[2] The treatment of keloids is a great challenge, since surgical excision alone results in high recurrence rates (>60%) and even growth stimulus following treatment.[1] To date, several treatment modalities exist, but not a single treatment option has proven widely effective.[3,4] First line non-surgical treatment options include silicone sheeting, pressure therapy, intralesional corticosteroids and intralesional 5-fluorouracil.[3,5] The evidence for effectiveness of silicone sheeting and pressure therapy remains limited.[5] Intralesional corticosteroids and 5-fluorouracil have proven successful in reducing pain and pruritus, as well as decreasing scar volume. However, several painful treatment sessions are required and recurrence rates remain high.[4]

If these non-surgical treatment options fail, surgical excision with adjunctive radiation is considered the most effective treatment protocol.[6] It allows for complete scar eradication with low recurrence rates.[7] This therapy is however not suitable for children (<12yr) or keloids with cannot be closed primarily or are located near radio-sensitive organs such as the thyroid gland.[8]

Cryotherapy

Recently, a novel technique for the treatment of keloids was introduced offering a potential treatment modality between the current non-surgical and surgical treatment options: Intralesional (IL) cryotherapy.[11] For decades, liquid nitrogen has been applied externally to freeze and destruct keloids. However, numerous side effects such as hypopigmentation, blistering, delayed healing and infection were reported.[9,10] Furthermore, treatment of larger keloids required multiple cryotherapy sessions.[9,10]

To solve these problems, IL cryotherapy was introduced by Weshahy.[11] By using a hollow needle, a cryogen can be applied directly into the deeper dermis of the scar. In this way, all the pathological tissue will be frozen and destructed, creating a new scar without keloidal characteristics, while sparing the surface epithelium.[12] IL cryotherapy thus claims to enhance volume decrease while reducing the risk of hypopigmentation and other surface reactions.[13]

Working mechanism

The working mechanism by which cryotherapy *destructs* the keloid scar relies on two phases of cellular destruction: a physical phase and a vascular phase.[10,14] During the physical phase, rapid freezing causes direct cell injury through the formation of sharp ice-crystals. Moreover, the differential freezing of cell compartments leads to changing osmotic gradients and electrolyte imbalances causing irreversible cell damage. In the vascular phase, damage to and failure of the microcirculation leads to cell destruction through ischemic necrosis.[10,14]

The working mechanism by which cryotherapy *prevents* the keloid from recurring can be explained from two perspectives. Firstly, histological studies have shown cryotherapy to result in rejuvenation of the scar tissue. Freezing pathological scar tissue induces the differentiation of abnormal keloidal fibroblasts towards a normal phenotype.[15,16] In vitro, cryotherapy has been shown to result in normalizing the synthetic activity of keloid fibroblasts.[16] Post treatment, the ratio of type III to type I collagen is increased, resembling normal healthy tissue.[17]

Secondly, the absence of wound contraction following a freezing injury may be another explanation. In burns, wound contraction results in severe scarring and contractions.[18–20] After freezing of a wound however, no wound contraction is seen.[18–20] The cellular matrix remains following cryo-treatment and acts as a scaffold for cellular regeneration enhancing wound repair.[18–20] This may prevent recurrence, since high-tension locations are prone to keloids.[21]

Systematic review

Several studies investigating IL cryotherapy have been published. Remarkably, quite different outcomes were reported by these studies, especially in terms of recurrence rate and hypopigmentation incidence. In order to evaluate the effectiveness of IL cryotherapy, an overview of the current literature is required. This article discusses the findings of a systematic review to answer the question: Is IL cryotherapy an alternative to other well established keloid scar treatments? And in which role within the current spectrum of treatment modalities should it be positioned?

Methods

Search strategy

A comprehensive systematic review of the English-language literature was performed, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. PubMed and Embase were searched from inception to August 2014. The following terms were used as index terms or free-text words: ‘cicatrix’ or ‘scars’ (including synonyms and closely related words such as hypertrophic scar and keloid scar) and ‘cryotherapy’ or ‘cryosurgery’ or ‘cryoablation’ and ‘intralesional’. The full search strategies for PubMed can be found in the Supplementary Information. References of retrieved articles were scanned for additional studies. Inclusion criteria consisted of the following: 1) Any English-language randomized controlled trials (RCTs), controlled clinical trials (CCTs), prospective or retrospective cohort studies or pilot studies reporting treatment with IL cryotherapy for treatment of scars; 2) Studies including solely keloid scars or studies including hypertrophic and keloid scars. Exclusion criteria: 1) Experimental studies; 2) Studies assessing only patient satisfaction, without objective outcome measurements. In case of duplicate articles only one was included.

The article screening process was performed as follows: Three investigators (ML, AEB and JK) carried out the initial searches and two investigators (ML and AEB) independently reviewed the studies for eligibility. Investigators were blinded to each other, meeting only to compare findings after completing the extraction process. Decisions about eligibility were resolved by discussion. Seventy-six potentially relevant studies were identified from the initial searches. Subsequently, 2 authors (ML and AEB) independently screened the full-text articles for eligibility using a standardized data extraction form with inclusion and exclusion criteria. Disagreement was resolved through discussion. This resulted in the inclusion of eight articles. See flow diagram 1.

Data extraction

One reviewer extracted data and a second review author verified the accuracy of the extracted data. Discrepancies in opinion about an article were reviewed and consensus was achieved through discussion. A standardized data form to capture the following information was used: 1) Study characteristics; 2) Study participants (including origin or Fitzpatrick score); 3) Scar characteristics (duration, location, etiology, previous treatments); 4) Study design (prospective/retrospective, follow-up duration; 4) Intervention, including type of device used and number of sessions; 5) Assessment/measurement method; 6) Study results, of which the recurrence rate was the main outcome. The data was summarized in an evidence table.

Methodological quality assessment

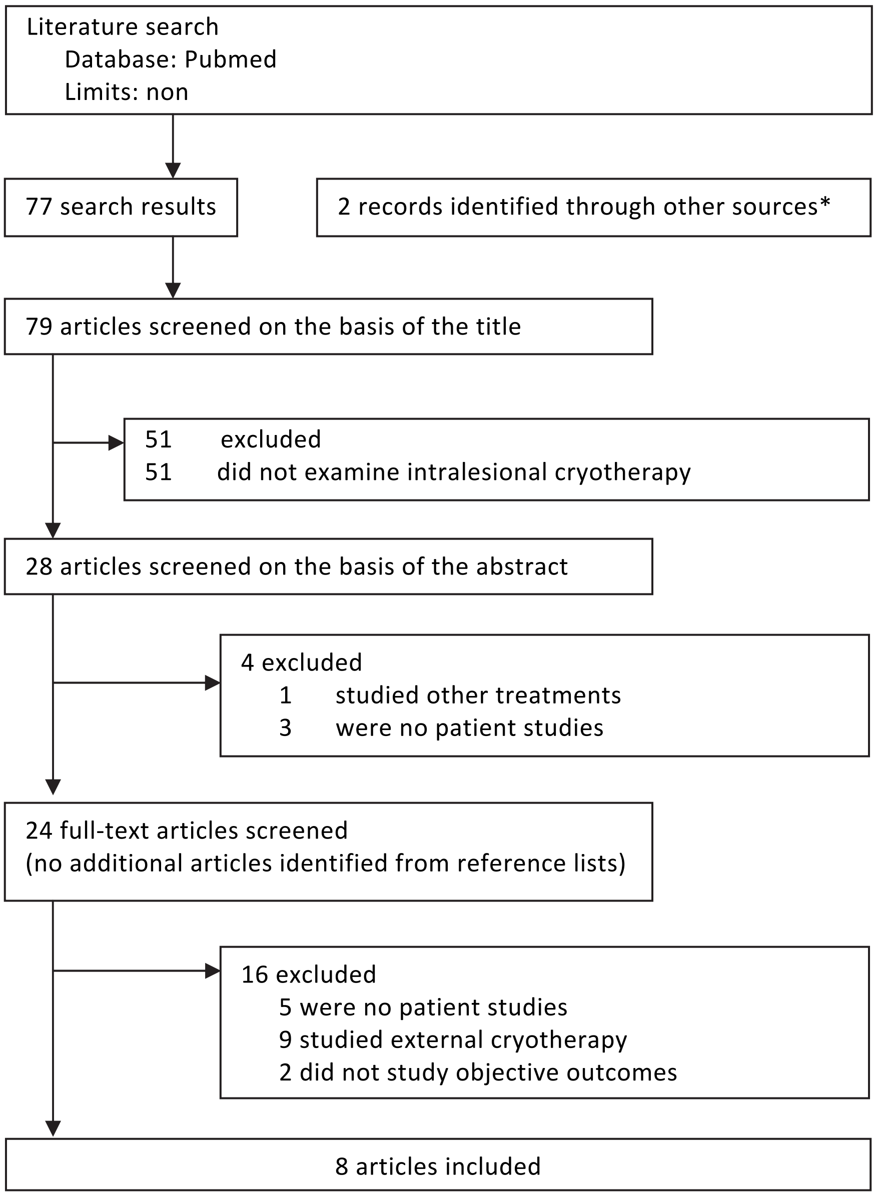
Heterogeneity in study design and outcome measures did not allow for quantitative pooling of data for meta-analysis. The level of evidence of the extracted studies was graded according to the American Society of Plastic Surgeons Rating Levels of Evidence.[22] This classification assigns each article to a corresponding level of evidence ranging from I (highest) to V (lowest). We classified a level II to prospective studies, which used a definition for inclusion of keloid scars and clearly defined their outcomes measurements including a definition for scar recurrence. Also, a minimum of a one year follow-up was required.[23,24] Finally, a practice recommendation was concluded based on the collected evidence according to the American Society of Plastic Surgeons Grade Recommendation Scale.[22]

Results

Study characteristics

Initial database searches identified 77 potentially relevant studies. Two studies accepted for publication, were also included.[25,26] Thus, 79 articles were screened on the basis of title, 28 on the basis of the abstract and finally, 24 full-text articles were analysed (see flow diagram 1). Excluded records did not investigate the effect of IL cryotherapy in patients or studied other treatment modalities as external cryotherapy. One excluded study reported solely subjective outcomes and one study investigated only the pigmentation change following treatment.[27,28] Finally, 8 articles met all inclusion criteria. A summary of the included studies is given in table 1.

Flow Diagram 1. Flow diagram of the Search and Selection Process according to PRISMA.*: 2 accepted records, included from the VU medical center research database [25,26]



Methodological quality

Most studies were prospective of nature, but did not differentiate between keloid and hypertrophic scars (n=2), lacked a definition for scar recurrence (n=6) or did not respect the minimum of a one year follow-up post-treatment (n=3). Therefore, only 2 studies were classified as level of evidence type II, 5 as type III and one as type IV. See table 1.

Patient characteristics

The sample size of the included studies ranged from 9 to 27 patients (mean: 17 ± 7.4). In total 136 patients with 160 lesions were treated. Follow-up ranged from 6 to 21.5 months with a mean follow-up of 14.5 ± 5.3 months. Patient's origin or Fitzpatrick skin type was described in 7 out of 8 studies; van Leeuwen et al., Stromps et al. and Zouboulis et al. included a mixed population including all races.[29,30] Har-Shai et al. included mostly Caucasian patients.[13] When looking at the Fitzpatrick skin type score, 2 studies included a Fitzpatrick (F) 1-2 skin type patient population, one study a F2-4 population and 4 studies included a F1-6 population.

Treatment modalities

The included studies used different treatment devices. Most studies used nitrogen-based cryodevices: Gupta and Kumar used simple lumbar puncture or hypodermic needles.[12] Zouboulis et al. used a flexible metallic cryoprobe stem and Weshahy and Abdel Hay designed 'Weshahy cryoneedles'.[30,31] Har-Shai et al. used a disposable 14-gauge double-lumen cryoneedle called the CryoShape® (Etgar Group International Ltd, Kfar Saba, Israel).[13] Van Leeuwen et al. used the same device, but also tested an argon gas based cryoneedle called IseSeed® (Galil Medical, Yokneam, Israel).[25,26] Most studies evaluated treatment outcomes after a single cryosession, but some used up to 10 sessions. Finally, Weshahy and Abdel Hay[31] and Stomps et al.[29] combined IL cryotherapy with adjuvant therapy with silicone sheeting and triamcinolone injections, respectively.

Recurrence and volume decrease

Scar recurrence following treatment ranged from 0 up to 24%, with a mean of 7.6±10.1%. Weshahy and Abdel Hay reported small recurring scars (0.5-1cm^3) at the periphery in 12% of the scars, which disappeared gradually through repeated IL steroid injections.[31] Zouboulis et al. reported a volume increase following treatment in two patients (20%), but did not reported any recurrences.[30] One study did not reported the incidence of scar recurrence.[12]

Volume decrease was measured differently by the included studies: van Leeuwen et al. made a mold of the scar using dental putty. Thereafter, the mold was filled with plaster and weighted to obtain the volume.

Table 1. Summary of the included studies

Study	Study Type	N (P/S)	Scar type	Skin type	Follow-up (range)	Cryodevice
Gupta et al., 2001	P	12/12	K	NS	7-12m	Nitrogen based, multiple lumbar puncture and/or hypodermic needles
Har-Shai et al., 2003	P	10/12	K+H	F1-3	18m	Nitrogen based, double-lumen cryoneedle
Zouboulis et al., 2004	Pi	10/10	K	F1-6	6m	Nitrogen based, 20 gauge metallic cryoneedle
Stromps, Har-Shai et al., 2014	R	21/32		F1-6	18.62 m (12-24)	Nitrogen based, double lumen cryoneedle + silicone sheeting
Har-Shai, Zouboulis et al., 2006	P	30/45	K	F1-3	15m	Nitrogen based, double-lumen cryoneedle
Weshahy et al., 2012	P	22/25	K+H	F2-4	7-36m	Nitrogen based, Weshahy cryoneedles + rep. triamcinolone injections

Protocol	**Outcome measures**	**Assessment**	**Results**	**L/E**
Monthly ILC session: >5, <10	Volume reduction	NS	7/12 pts: ->75% 4/12 pts: -51-75% 1/12 pts: -40%	III
	Pain + Pruritus	NS	12/12 pts: disappeared	
	Hypopigmentation	NS	12/12 pts: persistent	
	Recurrence	NS	0%	
1 treatment session	Volume reduction	Putty water displacement method	-51% (p<0.002)	III
	Pain	Rating: 0-3	-78% (p=0.005)	
	Pruritus	Rating: 0-3	-62% (p=0.005)	
	Hardness	Rating: 0-3	-72% (p=0.002)	
	Redness	Rating: 0-3	-52% (p=0.01)	
	Recurrence	NS	0%	
	Hypopigmentation	NS	No permanent depigmentation	
Monthly session, >3, <6	Volume reduction	N/S	2/10 pts: -50%-100% 5/10 pts: -<50% 1/10 pts: 0% 2/10 pts: Increase	IV
	Hypopigmentation	N/S	40%	
	Recurrence	N/S	0%	
1-2 sessions: 2nd at 6mnd if no sign volume reduction	Volume reduction	Putty water displacement	Earlobe+ face: -89%	III
	Hardness	mGGS	Improvement, p<0.0012	
	Pain	mGGS	Improvement, p<0.0233	
	Discomfort	mGGS	Improvement, p<0.02	
	Recurrence	NS	0%	
	Hypopigmentation	NS	3/21 pts: temporary	
1 treatment session	Volume reduction	Putty water displacement	-67.4% (p<0.005)	III
	Pain	Rating: 0-3	-78% (p<0.004)	
	Pruritus	Rating: 0-3	-52% (p<0.016)	
	Hardness	Rating: 0-3	-72% (p<0.004)	
	Redness	Rating: 0-3	-83% (p<0.007)	
	Recurrence	NS	0%	
	Hypopigmentation	NS	No marked hypopigmentation	
1 ILC + < 8 ILCS	Volume reduction	Alginoplast + saline	- 94% (p<0.001)	III
	Pain	Rating: 0-3	-100% (p<0.001)	
	Pruritis	Rating: 0-3	-100% (p<0.001)	
	Hardness	Rating: 0-3	+92% (p<0.001)	
	Redness	Rating: 0-3	-81% (p<0.001)	
	Recurrence	NS	12%	
	Hypopigmentation	NS	21/35 pts: temporary 7/35 pts: persistent	

Table 1. Continued

Study	Study Type	N (P/S)	Scar type	Skin type	Follow-up (range)	Cryodevice
Van Leeuwen et al., 2014	P	27/ 29	K	F1-6	12m	Liquid nitrogen, double-lumen cryoneedle
Van Leeuwen et al., 2015	P	25/30	K	F1-6	12m	Argon gas to induce freezing and helium for thawing. 17 gauge cryoneedle

NS: not specified in study, ILC: Intralesions cryotherapy, ILCS: Intralesional corticosteroid injection N (P/l): Number of patients and lesions (patients/lesions). Scar Type: K: keloid, H: hypertrophic scar. Type of study: P: prospective, R: retrospective, Pi: pilot study. Skin Type: Fitzpatrick skin type; ranging from 1-6.

Har-Shai et al., Stromps et al. and Weshahy filled the mold with saline to measure scar volume.[13,29,31] Har-Shai et al. and van Leeuwen et al. reported a mean volume decrease ranging from 51.4-63% (range 16-100).[13,26] Stromps et al. reported a 89% volume decrease for facial scars, while presternal scars showed only 47% volume decrease.[29] See also table 1.

Elasticity

A 36% and 57% elasticity increase was showed in two studies, in which an objective measurement devices were used.[25,26] Other studies measured improvements ranging from 71-92%, using non-specified elasticity or hardness measurement methods.

Pigmentation and redness

Har-Shai et al. reported no permanent or marked hypopigmentation in Caucasian patients following treatment in both studies.[13,17] In contrast, Van Leeuwen et al. reported hypopigmentation in most scars following treatment in a patient population including patients of all Fitzpatrick skin types. Although hypopigmentation recovered in the majority of scars, persistent hypopigmentation was seen after 12 months in both studies (31% [26]and 37%[25] resp.).

Protocol	Outcome measures	Assessment	Results	L/E
1-2 sessions: 2nd at 6mnd if <50% vol. decrease	Volume reduction	Putty plaster displacement	-63%, r:16-100, p<0.01	II
	Redness	Dermaspectrometer	Returned to pre-treatment value, after initial increase	
	Elasticity	Cutometer	+57%	
	Pain	POSAS	-45%	
	Pruritis	POSAS	-28%	
	Overall improvement	POSAS	Doctor: +24% Patient: +52%	
	Recurrence	*	24%	
	Hypopigmentation	>1% surface	9/29 pts: persistent	
	Complications		7.5% wound infection	
1 session	Volume reduction	Putty plaster displacement	- 62.1%	II
	Redness	Derma Spectrometer	No change	
	Elasticity	Cutometer	+36%	
	Pain	POSAS	-35%	
	Pruritis		-33%	
	Overall Scar appearance	POSAS	doctors: + 9% patients: +32%	
	Recurrence	*	17%	
	Hypopigmentation	>50% surface	11/30 pts: persistent	
	Complications		10% wound dehiscence	

L/E: Level of Evidence. According to American Society of Plastic Surgeons Rating Levels of Evidence and Grading recommendations for Diagnostic studies. V: lowest level of evidence, I: highest.
* judgment of recurrence, defined as a growing, pruritic, nodular scar as described by Cosman and Wolff.[24]

Other studies did not clearly described whether the hypopigmentation persisted and to what degree. Two studies measured an increase in redness following treatment. After 12 months, however, redness returned to pre-treatment values.[25,26] Other studies reported redness to decrease with 52-83% following treatment.

Scar assessment

Subjective scar evaluation improved in all studies, although three studies did not quantify their results. Complaints of pain decreased with a mean of 52.5 ± 18.4% (range 35-78) after treatment and itching decreased with 43.6 ± 15.8% (range 28-61), but never disappeared completely. Two studies showed an improvement according to patients and doctors using the Patient and Observer Scar Assessment Scale. Patients and doctors scored an improvement of 32% and 9% following treatment with the argon gas-based device and a 52% and 24% improvement with the liquid nitrogen-based device, respectively.[25,26]

Fitzpatrick skin types

Most studies treated Caucasian (F1-2) patients. Two studies included a F1-6 patient skin type population and reported some remarkable differences in outcomes between patients with diverse skin types.[25,26] Firstly, persistent hypopigmentation was mainly seen in F 4-6 skin type patients. Compared to F1-2 patients, there was a significantly higher incidence of hypopigmentation in F5-6 patients (p= 0.02).[17]

Secondly, F1-2 scars showed a statistically significant greater improvement in subjective scar evaluation compared to F5-6 scars, as measured with the POSAS by doctors (p=0.03) and patients (p=0.04).[17] In addition, high odds ratios were seen for recurrence in F3-4 (56%) and F5-6 (66%) patients, compared to F1-2 patients.

Complications

All studies reported mild to moderate post-operative pain with local oedema and superficial necrosis in the first weeks following treatment. Wound infection and wound dehiscence was reported by two different studies, in 7.5% and 10% of the patients respectively. Both were successfully treated conservatively.[17,21]

Discussion

Intralesional (IL) cryotherapy is designed to destroy the core of the keloid, while at the surface, cells including melanocytes are much less affected.[17] As such, IL cryotherapy aims to enhance volume reduction, decrease recurrences while minimizing the risk of hypopigmentation. Although studies were initially promising, recent studies provided different insights. This manuscript addresses the question whether this treatment is an alternative to other keloid scar treatments.

Treatment protocol

Although most studies were prospective, only two were classified as level II evidence studies. Other studies were graded with a lower classification due to various limitations: Firstly, most studies did not meet the minimum criterion of a one year follow-up.[32] This is essential to reliably assess scar recurrences. Secondly, many studies did not use a definition to distinguish keloid and hypertrophic scars. This is relevant, since hypertrophic scars have a better prognosis than keloid scars.

Finally, to quantify outcome measurements, validated and reliable subjective and objective scar measurement tools should be used. Examples are the Cutometer for scar elasticity[33], the Dermaspectrometer for scar colour[34] and the Patient and Observer Scar Assessment Scale for subjective scar assessment by doctor and patient.[35,36]

The use of definitions and measurement devices will enhance reliable assessment of treatment outcomes and can make comparison of study results possible.

Devices

A number of devices have been described for the treatment of keloid scars with IL cryotherapy. However, only two devices are currently commercially available; a liquid nitrogen-based[13] and an argon gas-based device.[25] The liquid nitrogen-based device is a double-lumen 14 gauge cryoneedle. The cryoneedle is connected via an elongation tube to a simple Dewar cylinder, in which liquid nitrogen is stored. After pressure has built up inside the cylinder, the liquid nitrogen is forced through the cryoneedle, which freezes along the entire track.

Van Leeuwen et al. reported freezing capacity problems with the above described liquid nitrogen system.[25] When treating large or multiple keloid scars, elongated or even dysfunctional treatments were observed. Therefore, they tested another and novel system based on argon gas.[25] With this system, high pressurized argon gas (300 bar) is led through a 17 gauge disposable cryoneedle. This results in a rapid freezing process only at the tip of the needle (as opposed to the nitrogen-based cryoneedle which freezes along the total track of the needle). In addition, the freezing process is monitored and can be adjusted to control the procedure. Also, a thawing cycle can be induced via the same needle using helium gas, to allow for the gentle removal of the cryoneedle from the frozen tissue. It should however be mentioned that the costs of the argon gas-based system exceed the liquid nitrogen-based device significantly.

Volume and recurrences

Most studies reported >50% volume decrease following treatment. However, on average, there was no complete scar eradication in the included studies. Even after a maximum of ten sessions of IL cryotherapy in the study of Gupta and Kumar, no complete eradication was achieved.[12] It is therefore questionable whether IL cryotherapy will achieve the same results as with excision of the scar, even after multiple sessions. More likely, IL cryotherapy will result in an ‘acceptable’ volume reduction as with non-surgical treatments like corticosteroid injections.

While some studies did not report any recurrences, others reported a recurrence ratio up to 24%. Two factors can account for this inconsistency: Firstly, some studies did not respect the minimum of a one year follow-up, as discussed above. Secondly, most studies reporting low or no recurrence ratios, included only Caucasian patients. In contrast, two studies reported increased odds ratios for recurrence in F3-4 and F5-6 patients, compared to F1-2 patients.[25,26] The relation between recurrence and a Fitzpatrick score of more than 3, is described by other authors as well.[37] Therefore, it is important to include a patient population consisting of all Fitzpatrick skin types.

Persistent hypopigmentation

IL cryotherapy was designed to overcome pigmentation disorders associated with external cryotherapy. Several authors, therefore, encouraged the use IL cryotherapy for dark-skinned individuals suffering from keloid scars.[27,28] In a controlled study, Van Leeuwen et al. demonstrated a significant higher incidence in F5-6 patients compared to F1-2 patients in a patient population consisting of all Fitzpatrick skin types.[26] Other studies also confirmed the incidence of persistent hypopigmentation following IL cryotherapy. However, it was not always reported clearly whether the hypopigmentation remained and to what degree. In our clinical center, we experienced that any hypopigmentation is considered as very disturbing or even traumatic for patients. Since clinical studies reported hypopigmentation in patients with a Fitzpatrick of more than 3, we advise to use IL cryotherapy in those patients only when the scar is non-visible (for example: retro-auricular).

The role of IL cryotherapy in current scar therapies

This systematic review showed IL cryotherapy to be a promising treatment modalities for the treatment of keloid scars in terms of volume reduction and alleviation of pain and pruritus. However, on average, no complete scar eradication is attained and scar recurrence is seen. Also, persistent hypopigmentation remains problematic in non-Caucasian patients. These issues raises the question whether IL cryotherapy is a viable treatment alternative to other established scar treatments.

To make IL cryotherapy a worthwhile treatment, novel systems or adjustments of the existing systems are required to obtain complete scar eradication, lower the recurrence rates and control hypopigmentation. Also, high-quality randomized studies will have to generate stronger evidence proving the effectiveness of IL cryotherapy and its safety in the different Fitzpatrick skin type groups. The evidence provided by the studies included in this review proved limited and inconsistent in terms of effectiveness, resulting in a grade C practice recommendation for IL cryotherapy to date. High-quality randomized studies are required to generate stronger evidence proving the effectiveness of this technique.

Ultimately, IL cryotherapy could be an addition to the existing keloid scar treatments: 1) If non-surgical techniques have failed. 2) As combination therapy with nonsurgical therapies as steroid injections[12] or silicone gel sheeting.[29] 3) As alternative for excision with adjuvant irradiation in case radiotherapy is not available or the patient (<12yr) or keloid (size, anatomical location) is not suitable for radiation therapy. 4) In a specific subgroup of patients seeking for alleviation of pain and pruritus rather than complete scar eradication.

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Chapter 5

Intralesional cryotherapy for treatment of keloid scars; a prospective study

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Abstract

Background

Intralesional (IL) cryotherapy is a novel treatment for keloid scars, in which the scar is frozen from inside. Published results are promising, but the treatment has only been tested in a Caucasian patient population. Therefore, we evaluated IL cryotherapy in a patient population including different Fitzpatrick (F1-6) skin types.

Methods

This prospective multicenter study with a 1 year follow-up included 27 patients with 29 keloid scars. IL cryotherapy was administered with a disposable liquid nitrogen based device called Cryoshape. Scar assessment was performed using the Patient and Observer Scar Assessment Scale (POSAS) and four objective devices to determine scar *color*, scar *elasticity*, scar *volume* and patient's *skin type*.

Results

Keloid scars showed an average volume decrease of 63% (range of 16-100%) after 12 months, compared to baseline $p<0.01$. Recurrence was seen in 7 keloids (24%) and hypopigmentation recovered in 69% off all keloid scars within 12 months. Scar assessment with the POSAS showed an overall improvement according to both doctors and patients. In addition, complaints of pain and itching were alleviated.

When analyzing the results per Fitzpatrick skin type, Afro-American patients showed a higher incidence of persistent hypopigmentation ($p=0.02$).

Conclusions

Intralesional cryotherapy for the treatment of keloid scars shows favorable results in terms of volume reduction and alleviated complaints of pain and pruritus. However, no complete eradication was obtained in some cases and recurring scars were seen. In addition, persistent hypopigmentation proved a problem in non-Caucasian patients.

Introduction

Keloids result from an abnormal healing response of injured skin. Besides aesthetic disfigurement, keloids can cause major physical complaints of pain and pruritus, hence impairing the quality of life of the patient.[1–4] Treatment is difficult, with high recurrence rates and even growth stimulus following treatment as the main issues.[5] Physicians are therefore still in search for an optimal treatment. Cryotherapy, also called cryoablation or cryosurgery, is one of the options.[6,7] For decades, liquid nitrogen has been applied externally to keloid scars, but multiple side effects such as hypopigmentation, blistering, delayed healing and infection were reported.[6,7] Another problem was the difficulty of reaching deeper dermal sections of the scar, which resulted in a high recurrence rate and limited its use to small scars only.[6,7] In 1993, Weshahy devised a solution by introducing a new method that applied liquid nitrogen intralesionally with a hollow needle: Intralesional (IL) cryotherapy.[8] This allowed the deeper dermis to be reached, preventing recurrence. Also, the epithelium was affected less, thus reducing hypopigmentation. Gupta et al. published a similar method with multiple hypodermic needles in 2002[9] and Har-Shai et al. eventually refined this technique by introducing a disposable cryotherapy instrument called Cryoshape.[10,11] In their first study, scar volume was reduced by an average of 51.4% after a single session of IL cryotherapy with no major complications or recurrence after 18 months.[11] However, only 10 Caucasian patients were included in this study. Since keloid incidence and rates of recurrence and pigmentation problems are higher in Afro-American individuals[12,13], a study including patients of different Fitzpatrick skin types (F1-6) is desirable. Therefore, we evaluated IL cryotherapy in a prospective multicenter study with a patient population including all Fitzpatrick skin types. Scar assessment was performed using the Patient and Observer Scar Assessment Scale and four objective devices to determine scar *colour*, scar *elasticity*, scar *volume* and patient's *skin type*.[14–16]

Patients and methods

Patients

All patients visiting the plastic surgery department of the VU University Medical Center (Amsterdam, the Netherlands) and the Red Cross Hospital (Beverwijk, the Netherlands) between 2009 and 2011, meeting inclusion criteria, were included in a prospective study evaluating the use of IL cryotherapy. Inclusion criteria were: 1) Patients with keloid scars. Keloids were distinguished from hypertrophic scars based on the clinical judgment of their growth pattern and defined as a fibroproliferative disorder of the skin that grows beyond the boundaries of the original wound or had an unrecognized origin, as described by Ogawa.[17] 2) The period between previous treatment and IL cryotherapy covered a minimum of 12 weeks.

3) Patients of all Fitzpatrick skin types.[18] 4) Patients older than 10 years of age. Exclusion criteria were: pregnancy and diabetes mellitus and patients with collagen diseases.

Patient characteristics are listed in table 1: Thirty patients were included, 1 patient died (non-related cause) and 2 patients were lost in follow-up. Twenty-seven patients with 29 keloids completed the one year follow-up. The study group included 8 males and 19 females with a mean age of 31.7 years (range 12-71). Mean scar duration was 6 years (range 1-21) and mean scar size was 3.9 cm^3 (range 0.2-17). Out of the 27 patients, 2 patients with each 2 keloid scars were included. These scars had different etiologies, durations and anatomical locations and were therefore analyzed as separate entities. Patients were subdivided according to Fitzpatrick score in three groups: Fitzpatrick (F) 1-2; Caucasian patients, F3-4; Mediterranean/Asian patients and F5-6; Afro-American patients.

Table 1. Patient characteristics

N	Age (yr)	Sex	Location	Pre	Cause	Scar Duration (yr)
Fitzpatrick 5/6						
1	40-60	F	Other	None	Surgery	2
2	10-20	F	Ear	ST	Surgery	2
3	10-20	M	Face	None	Infection	4
4	40-60	M	Face	None	Trauma	15
5	40-60	M	Sternum	None	Trauma	15
6	20-40	F	Back	None	Surgery	6
7	20-40	M	Ear	ST	Surgery	7
Fitzpatrick 3-4						
8	60+	F	Sternum	ST	Burn	3
9	20-40	F	Ear	None	Trauma	1
10	40-60	F	Extremity	ST	Surgery	9
11	10-20	F	Ear	None	Surgery	1.5
12	40-60	F	U	None	Surgery	4
13	40-60	F	U	None	Surgery	4
14	60+	M	Sternum	Excision	Surgery	4
15	10-20	F	Ear	ST	Surgery	4
16	10-20	F	Ear	ST	Surgery	5
17	40-60	M	Back	ST	Trauma	3
18	10-20	M	Ear	None	Surgery	3
19	10-20	F	Ear	ST	Surgery	5
20	40-60	F	Other	ST	Surgery	4
21	40-60	F	U	None	Surgery	21
22	40-60	F	U	None	Surgery	14
23	20-40	M	Face	ST	Other	1
24	10-20	F	Ear	ST	Surgery	3
Fitzpatrick 1-2						
25	10-20	M	Back	ST	Infection	1
26	40-60	F	Ear	ST	Surgery	1
27	20-40	F	Ear	ST	Surgery	9
28	20-40	F	Back	ST	Burn	10
29	10-20	M	Back	ST	Surgery	4

N: scar number, Sex: M: male, F: female, Location: U; umbilicus. Pre: Pre-treatment, ST: Intralesional steroids.

Surgical procedure

Skin surface was disinfected and the scar was anesthetized locally with bupivacaine 0.5% with adrenaline using an extralesional approach. Next, the cryoneedle (CryoShape, Etgar Group International Ltd, Kfar Saba, Israel) was introduced longitudinally at mid-height and mid-width of the scar in a forward rotary movement until the distal edge was penetrated. The surrounding skin was covered with sterile gauze to prevent the cryoneedle from freezing healthy skin. The needle was connected via an elongation tube to the cryogen source (Cry-AC, type: B-700, Brymill Cryogenic Systems, Ellington, CT) which was filled with liquid nitrogen. During the freezing process, two ice circles appeared at the proximal and distal cryoneedle penetration sites. With larger keloids, multiple insertions were required to freeze the scar completely. The simultaneous use of multiple needles was also possible (figure 1 and 2).

Figure 1. Intralesional cryotherapy.

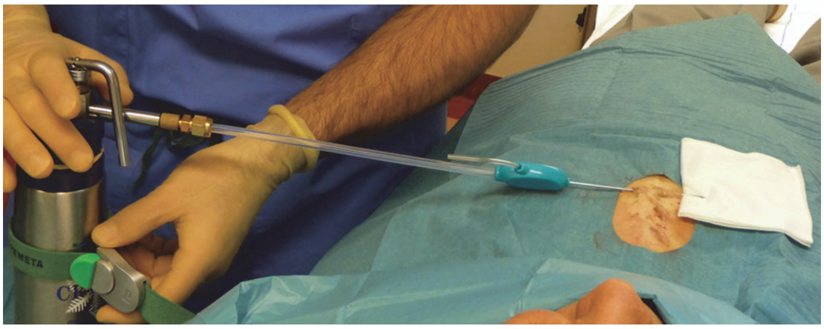


Figure 2. Intralesional cryotherapy (A).The use of needles simultaneously was also possible.

Once the scar was clinically completely frozen, the cryoneedle was gently removed. Afterwards, sterile dressings were applied and patients were instructed to wash the treated scar with water daily.

Evaluation

All patients were consulted by both surgeon and researcher before treatment, gave informed consent and agreed with the follow-up. Two independent researchers (ML and MW) assessed the scars. Three surgeons performed the surgical procedure, which was always strictly performed as described above. Before treatment, patients' personal data and details on the origin of the scar were documented in a standardized Case Report Form. Patients were seen at baseline and then at 3, 6 and 12 months post treatment. A second treatment always took place 6 months following the first treatment, if a scar volume decrease of less than 50% was obtained. Evaluation consisted of: 1) Skintype, classified into Fitzpatrick score by patient and physician.[18] 2) Comparison of photographs, taken before treatment and at each of the follow-up consultations. 3) Judgment of recurrence, defined as a growing, pruritic, nodular scar as described by Cosman and Wolff.[19] 4) Judgment of persistent hypopigmentation was defined as: "Any hypopigmentation of the treated scar surface after 12 months". 5) Scar elasticity, measured in two parameters: *extension* and *elasticity* (Cutometer Skin Elasticity Meter 575, Courage and Khazaka Electronic GmbH, Cologne, Germany).[20] 6) Redness (*erythema*) and pigmentation (*melanin*), which were measured using the DermaSpectrometer (Cortex Technology, Hadshund, Denmark).[21] 7) Scar *volume*, determined by creating a mold of the scar with dental putty (Cavex CA37, Alginate impression material, CAVEX Holland, the Netherlands). 8) Subjective scar evaluation, performed by two experienced medical doctors and the patient using the Patient and Observer Scar Assessment Scale (POSAS). Both the patient and observer scale consists of six items that was scored using a 10-step score, in which 10 reflected 'worst scar imaginable' and 1 indicated 'normal skin'.[14-16,22] The study was approved by the medical ethical council of VU University in the Netherlands.

Statistical analyses

Data were analyzed using SPSS version 20.0 (SPSS, Inc. Chicago, Ill). After being tested for normality, a paired *t* test was applied. Non-normally distributed data were analyzed by means of the Mann-Whitney test. The significance criterion for all tests was set a 0.05. Odds ratios and correlations were tested according to a linear or bivariate regression.

Results

At 12 months follow-up, the objective parameters and the Patient and Observer Scar Assessment Scale improved for keloid scars (figure 3). Twenty scars received 1 treatment and 9 scars received a second which always took place 6 months following the first treatment.

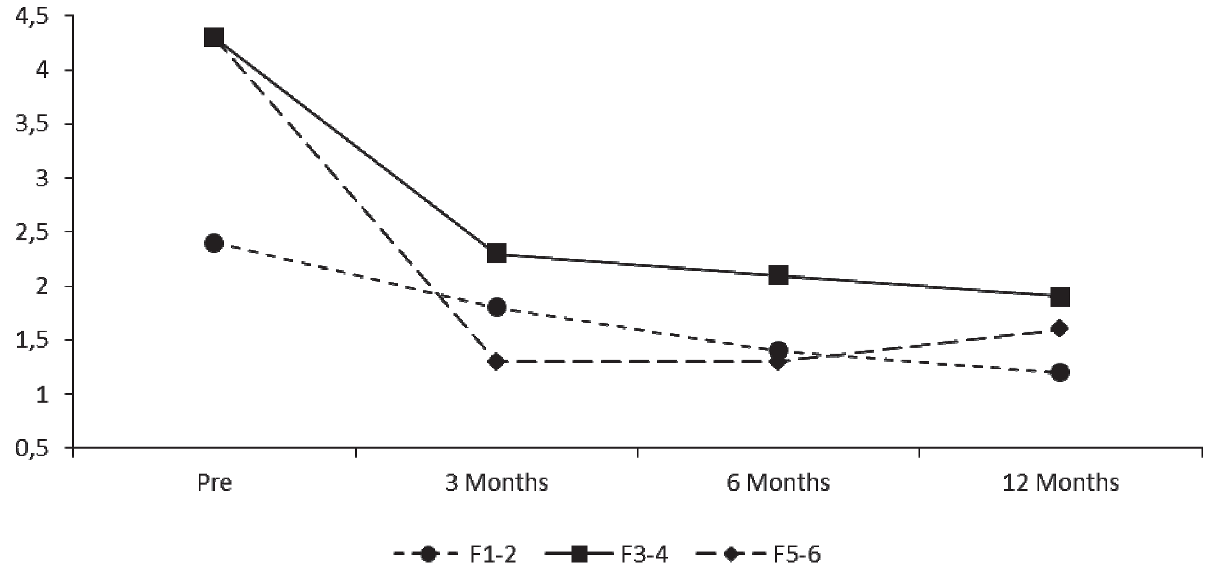
Figure 3. Outcome of Intralesional cryotherapy of a preauricular keloid: before (above, left and above, center) and 1 week (above, right), 6 weeks (below, left), and 12 months (below, center, right) after a single treatment. No recurrence was seen.

Volume and recurrence

The average volume of the nonrecurring keloid scars decreased from 4.3 ± 0.9 cm^3 before treatment, to 1.6 ± 0.5 cm^3 after 12 months of treatment, indicating a mean volume reduction of 63% (range 100-16%) compared to baseline values, p<0.001 (figure 4).

Interestingly, recurring scars showed the same volume decrease as the nonrecurring scars, but the volume started to increase by 6 months after treatment (0M: 2.9 ± 0.9, 6M: 1.6 ± 0.9, 12M 2 ± 0.6) (See figure 4). In total 7 out of 29 keloid scars (24%) recurred within 12 months after treatment.

Figure 4. Scar volume measured in cm^3 before and 12 months after treatment. Keloid scars subdivided according to Fitzpatrick (F) score into F1-2; Caucasian patients, F3-4; Mediterranean/Asian patients, F5-6; Afro-American patients.



Pigmentation

Most treated areas showed hypopigmentation after treatment, but repigmentation was seen in 69% of the cases. Still, persistent hypopigmentation was observed in 9 out of 29 keloid scars after 12 months (figure 5). Compared to baseline values, these scars showed an average pigmentation decrease of 45% measured in melanin in both hypopigmentation scars (0M; 89 ± 3.8, 12M; 50 ± 8.8, p=0.04 and ormal scars (0M; 40 ± 4.6, 12M; 37 ± 5.7) (figure 6).

Redness

Scar redness, as measured in erythema index, demonstrated a 56% increase after 3 months compared to before treatment. However, after 12 months, redness had returned to the same level as before treatment (erythema; 0M; 12.2 ± 6.4, 3M; 19 ± 9.7, 12M; 14 ± 6). Notably, scar redness was highly present in recurring scars (0M: 14 ± 4, 12M: 16 ± 2.5).

Figure 5. Hypopigmentation after treatment of a keloid scar loated on the thorax after 1 year.

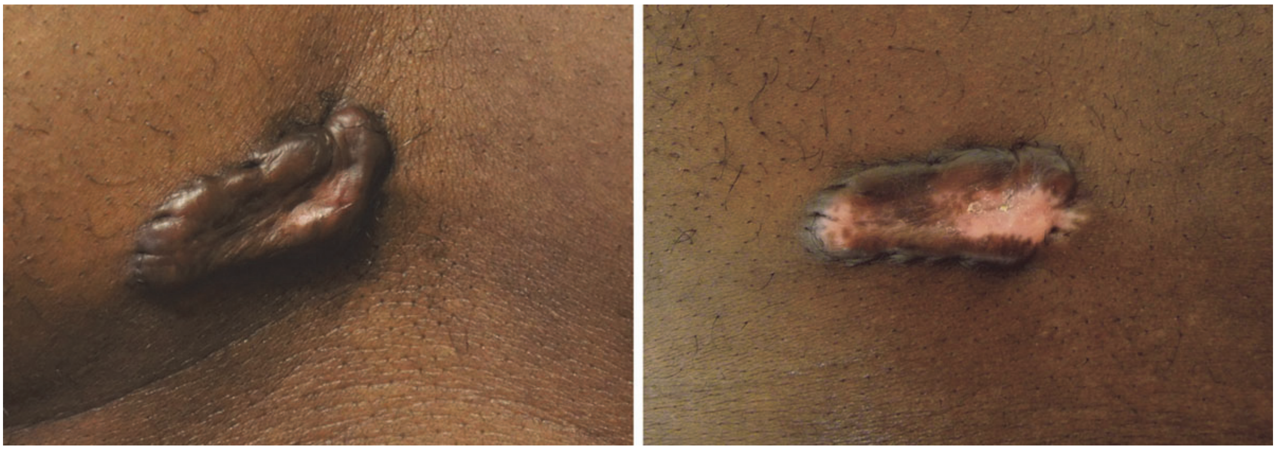
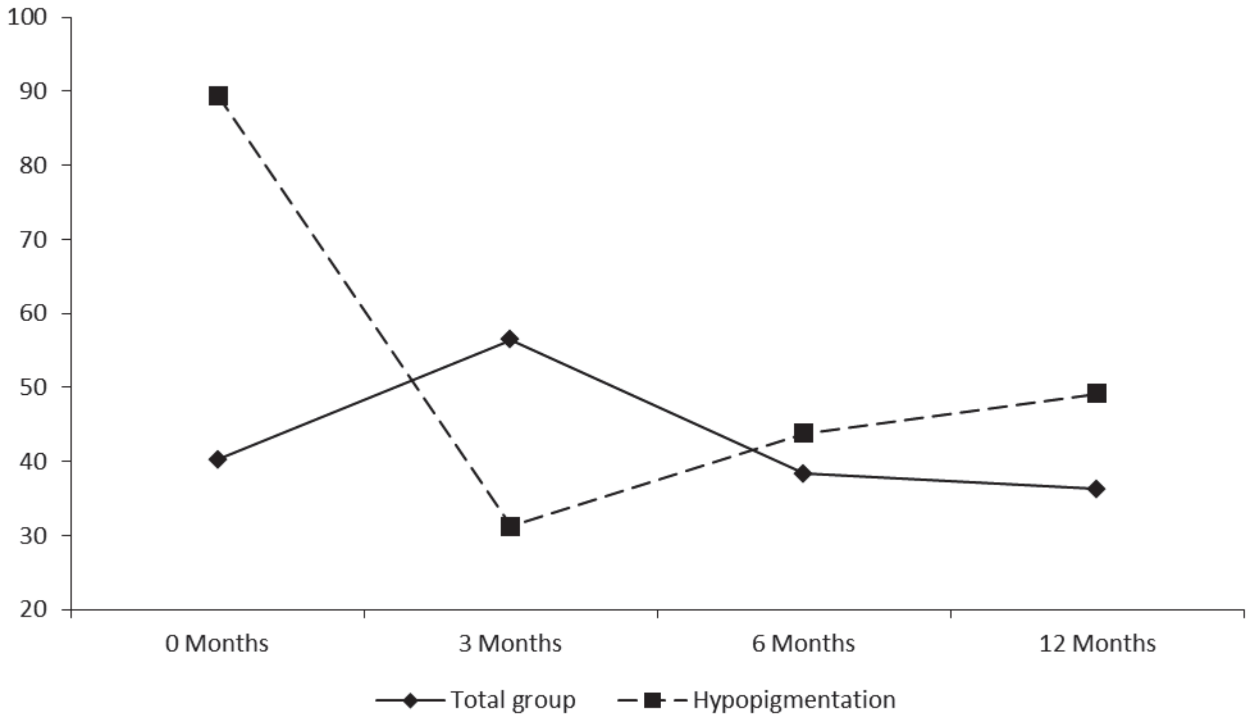


Figure 6. Pigmentation in melanin index before and after treatment (3, 6 and 12 months).



Elasticity

Scar elasticity was measured as the extent of skin stretching in millimeters, reflecting skin thickness and rigidity.[20] Elasticity improved in the successfully treated scars with 57% compared to baseline (0M: 0.28 ± 0.1, 12M: 0.4 ± 0.1, p= 0.02). In contrast, the elasticity of recurring scars deteriorated with 50% (0M: 0.4 ± 0.25, 12M 0.22 ± 0.06).

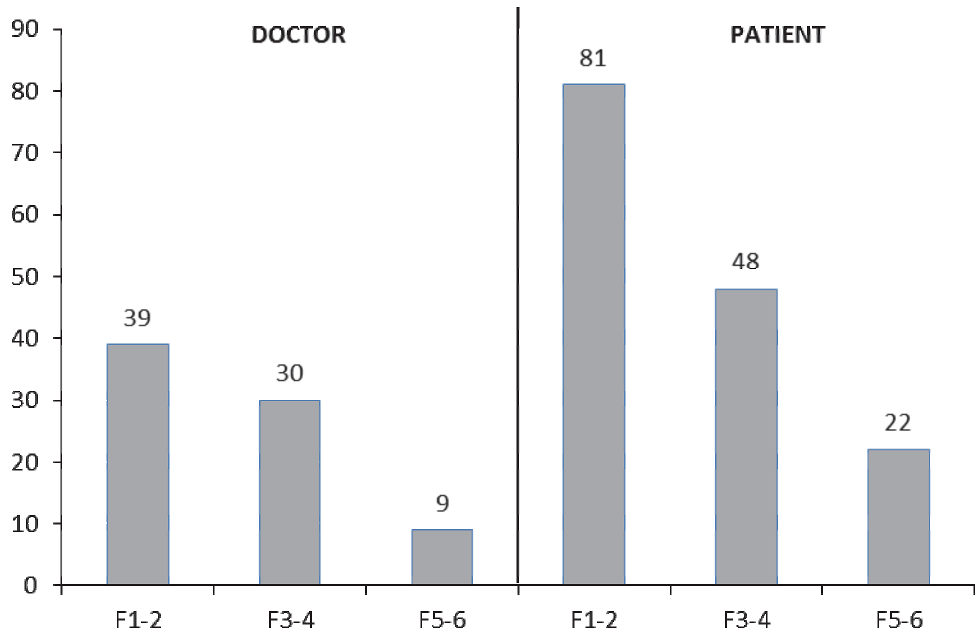
Scar assessment

The Patient and Observer Scar Assessment Scale (POSAS) improved according to both doctors and patients (figure 7):

Doctors scored a 24% improvement on the total POSAS score after 12 months, compared to baseline. When looking to the different parameters of the POSAS, a positive trend in favour of the treatment was scored. However, no statistical significance was reached.

In line with the doctors assessment, patients showed a 52% ($p<0.01$) improvement after 12 months compared to baseline. In addition, patients scored 45% less pain and 28% less itching 12 months after treatment, although not statistical significant.

Figure 7. Patient and Observer Scar Assessment Scale improvement in percentage 12 months after treatment compared to before treatment. Total POSAS score was stated by both doctor and patient and subdivided according to Fitzpatrick (F) score into F1-2; Caucasian patients, F3-4; Mediterranean/Asian patients, F5-6; Afro-American patients.



Fitzpatrick skin type

All results were analyzed into different Fitzpatrick scores (table 2). Two statistical significant differences were measured: Firstly, the incidence of persistent hypopigmentation was significantly higher in F5-6 patients compared to F1-2 patients, $p= 0.02$. All patients suffering from persistent hypopigmentation had a Fitzpatrick skin type of more than three (4 patients F3-4, 5 patients F5-6). Secondly, F1-2 scars showed a statistically significant higher improvement compared to F5-6 scars, as measured with the total POSAS scored by doctors ($p=0.03$) and patients ($p=0.04$).

Table 2. Measurements stated per objective device and divided per Fitzpatrick skin type

	Fitzpatrick Skin Type 1-2		Fitzpatrick Skin Type 3-4		Fitzpatrick Skin Type 5-6	
	0 Months	**12 Months**	**0 Months**	**12 Months**	**0 Months**	**12 Months**
Recurrence	-	1/5	-	4/17	-	2/7
Hypopigmentation	-	0/5	-	4/17	-	5/7
Volume (cm^3)	2.3 ± 2.6	0.8 ± 1.3	4 ± 4.5	1.7 ± 2	4.5 ± 2.3	1.9 ± 1.4
Redness	5.8 ± 2.2	8.9 ± 4.3	12.7 ± 5	15.7 ± 6.3	17.2 ± 7.4	13.4 ± 2.1
Pigmentation	33.9 ± 4	32.4 ± 3.3	57.2 ± 21	50.7 ± 22	80.7 ± 19	60 ± 26
Elasticity	0.15 ± 0.01	0.28 ± 0.02	0.13 ± 0.09	0.4 ± 0.3	0.15 ± 0.04	0.48 ± 0.05
POSAS						
Patient:						
- Painful	5 ± 2.6	2 ± 1.7	3.2 ± 3	2 ± 1.5	2.9 ± 2.9	1 ± 0.1
- Itching	3 ± 1.8	1.3 ± 0.6	4.8 ± 2.8	3.8 ± 2.7	4.7 ± 2.8	3.5 ± 3.5
- Colour	6 ± 1.4	5.7 ± 0.6	6.7 ± 2.4	5.8 ± 1.7	6 ± 1.9	5.5 ± 2.1
- Stiffness	6 ± 2	3 ± 1.7	8.1 ± 1.8	4.4 ± 2.1	5 ± 2.5	2 ± 0.3
- Thickness	7.3 ± 2.2	4.3 ± 2.1	7.6 ± 2	4.4 ± 2.1	6.9 ± 2.3	7.5 ± 0.7
- Irregularity	6.6 ± 0.9	3.3 ± 1.5	6 ± 2.7	4.3 ± 2	6.3 ± 3.3	6.5 ± 2.1
- Overall opinion	8.8 ± 0.8	1.7 ± 0.6	9 ± 0.4	4.7 ± 1.1	7.7 ± 2.7	6 ± 1.4
- total POSAS	31 ± 11.7	26 ± 1.4	36 ± 2.2	25 ± 3	31.8 ± 11.8	26 ±1.4
Doctor:						
- Vascularity	5 ± 0.1	3.7 ± 0.5	3.3 ± 2.1	3.1±2.1	2.9 ± 2.5	4.3 ± 1.3
- Pigmentation	2.3 ± 1.1	2.3 ± 1.5	4.5 ± 2.7	4.2 ± 2.4	5.3 ± 2.4	6.3 ± 1.5
- Thickness	5.3 ± 2.3	2.7 ± 1.1	5.4 ± 0.7	3.1 ± 0.7	6.3 ± 2.5	3.8 ± 1.3
- Relief	1.7 ± 0.6	2 ± 1	2.8 ± 0.4	2.9 ± 1.4	4.3 ± 1.4	3.8 ± 1.8
- Pliability	4.7 ± 2.5	2.7 ± 1.2	6.2 ± 0.7	3.6 ± 0.6	6.3 ± 2.7	5 ± 2.2
- Surface area	5 ± 3	2 ± 1.7	5.9 ± 2.5	3.7 ± 0.3	5.2 ± 2.5	4.5 ± 1.3
- Overall opinion	6 ± 0.3	4.3 ± 0.9	5.5 ± 0.6	3.8 ± 1.4	5.7 ± 1.8	4.3 ± 0.9
- total POSAS	30 ± 10.3	27 ± 5.7	28 ± 8.8	19.9 ± 5.7	30.1 ± 10.3	27.5 ± 5.7

POSAS: Patient and Observer Scar Assessment Scale (as scored by doctor and patient). Fitzpatrick skin type: 1-2 (Caucasian patients), 3-4 (Mediterranean/Asian), 5-6 (Afro-American). Scar Elasticity was measured with the Cutometer, Scar colour measured in erythema and melanin index with the Dermaspectrometer and persistent hypopigmentation and recurrence were observed after 1 year.

In addition, it was noticed that 6 out of 7 patients with recurring scars had a Fitzpatrick skin type of more than 3, while only one patient was out of the F1-2 group. The odds ratio for recurrence was 56% higher in F3-4 patients and 66% higher in F5-6 patients, compared to F1-2 patients. All other parameters did not show significant differences between the Fitzpatrick skin types.

Complications, recurrence and additional treatment

All patients reported mild to moderate postoperative pain, for which analgesics were prescribed in advance. Postoperative crusting and blistering were seen in all patients, which lasted for a maximum of 3 weeks in the majority of patients. In 7.5% of the patients, wound infection was diagnosed or considered for which oral antibiotics were administered.

Discussion

Intralesional (IL) cryotherapy is an innovative treatment for keloid scars and was designed to solve the problems related to external cryotherapy.[8,9,11,23] As opposed to external cryotherapy, IL cryotherapy targets the deeper dermis, achieving greater volume reduction. In addition, recurrence is reduced and less hypopigmentation is seen.[9–11,23–26] It has proven effective in earlier studies, but only in small Caucasian patient populations.[9–11,23–26] Har-Shai suggested that: “IL cryotherapy may occupy an important position in the treatment of black and pigmented-skin populations, which present a high prevalence of keloids and who may benefit from a lower rate of post cryosurgery skin hypopigmentation”.[26]

This prospective multicenter study is the first study to present the results of IL cryotherapy in a patient population including all Fitzpatrick (F) skin types. It shows that: *A)* IL cryotherapy proved effective in decreasing scar volume and alleviating complaints of pain and pruritus.
B) Scar assessment improved for patient and doctors after 12 months, using the Patient and Obserever Scar Assessment Scale. C) Fitzpatrick type 5-6 patients have a high incidence of persistent hypopigmentation following IL cryotherapy and showed less satisfactory results compared to Fitzpatrick type 1-2 patients as measured with the POSAS.

Existing literature

Comparison of our results with the already published studies investigating IL cryotherapy is hardly possible because of the heterogeneity in study methods and outcomes of the published studies.[9,11,23,24] The mentioned studies included a relatively small Caucasian patient population and lacked a clear definition of recurrence and outcome measures. Also, they did not differentiate between keloid and hypertrophic scars and used different cryotherapy devices. Finally, follow-up was scored between 4-12 months.[9–11,23,24]

In our study, we used the definition for scar recurrence as set by Cosman and Wolff.[19] Moreover, in order to reliably investigate the effects of IL-cryotherapy and monitor recurrence, a follow up of a minimum of 12 months post treatment of a primal keloid should be respected.[27,28] In this study we used the Cryoshape needle, a dispensable FDA approved cryoneedle, which should be inserted into the core of the keloid, which differed from the device used by Gupta et al. and Weshahy et al.; Gupta et al. developed a simple device, using multiple LP/hypodermic (injection) needles covering the entire lesion.[9] Weshahy et al. placed several Weshahy cryoneedles at the base of the keloid only, resulting in destruction of the cellular elements and blood vessels of the base, hence less surface reaction.[8,24]

Volume

We found an average volume decrease of 63% for keloid scars and a recurrence rate of 24%. Other groups found similar results with respect to the volume decrease, but other recurrence ratios were reported. Har-Shai et al. found an average of 50-70% volume reduction in two of his studies with 10 patients in a 18 months follow-up using the same cryoneedle as in this study and reported no recurrence.[10,11] Weshahy et al. described an average of 93.5% volume reduction after 18 months with IL cryotherapy followed by corticosteroid injection in 22 patients with 12% 'small size' scar recurrences.[24] Gupta reported a volume decrease of 40-75% in 12 patients after 6-12 months and reported no recurrences.[9] Finally, Zouboulis et al. measured a volume decrease in 8 out of 10 patients.[23] He found a volume increase in 2 patients after 6 months, which in our opinion should be stated as a recurrence rate of 20%. Although all groups reported a volume decrease of more than 50%, on average, no complete volume reduction was achieved. One has to take into account that better results can be achieved by excision followed by brachytherapy in which complete scar removal is attained.[29]

In this study, a second treatment always took place 6 months following the first treatment, if a scar volume decrease of less than 50% was obtained. Other authors treated scars more often: Zouboulis et al. treated each patient with a minimum of 3 sessions and a maximum of 6 sessions once a month.[23] Gupta and Kumar repeated therapy depending on the responses and applied a maximum of ten sessions with intervals of 3-4 weeks.[9]

Recurrence rate

The recurrence rate as seen in this study is explained by the fact that a strict definition of recurrence was used, but also a minimum of one year follow-up was respected. Moreover, our study population was different in the amount of patients with different Fitzpatrick score; more recurring scars were seen in non-Caucasian patients, which were not present in the other studies. Also, we left hypertrophic scars out of this study, but they were treated with a high success rate in our hospital. The relation between recurrence and a Fitzpatrick score of more than 3, as seen in this study, was described by other authors as well.[27]

Physical complaints

Pain and pruritus are present in keloid scars and decrease the quality of life.[1–4] In our study, complaints of pain and pruritus decreased after treatment, as measured with the Patient and Observer Scar Assessment Scale.[16] Other studies confirmed these results: Har-Shai et al.[10] found the same reduction in complaints and Weshahy and Abdel Hay[24] and Gupta and Kumar[9] reported that pain and itching completely disappeared.

Hypopigmentation

After 12 months, persistent hypopigmentation was seen in 6 out of 29 keloids (21%). Although other groups reported pigmentation problems ranging from 0 to 30%, no persistent hypopigmentation was described.[9,11,23,24] These differences may be explained by the fact that our patient population included all Fitzpatrick skin types, while other groups included mainly Caucasian (F1-2) patients. In this study Afro-American (F5-6) patients had a statistically significant higher incidence of persistent hypopigmentation than Caucasian (F1-2) patients. This confirms that non-Caucasian individuals (F3-6) are more prone to pigmentation problems.[12,13]

This study also showed a higher erythema rate in F1-2 scars compared to F3-6 scars, but not significantly. This erythema may be explained by the new scar as created by IL cryotherapy. Since normal scar maturation can take up to one year, we don't expect scar erythema to consist after one year. This issue was explained to the patients before treatment.

Limitations

Firstly, a study performed in a randomized fashion would be favorable and therefore, our group participates now in a large randomized clinical study, and we hope to confirm the results as described in this study.[30] Secondly, this study respects the minimum of a one year follow-up, but suggestions have been made that a 2 or even 3 year follow-up is required in order to identify all recurrences.[31]

Conclusion

In conclusion, Intralesional cryotherapy for the treatment of keloid scars shows favorable results in terms of volume reduction and alleviated complaints of pain and pruritus. However, no complete eradication was obtained in some cases and recurring scars were seen. In addition, we conclude that non-Caucasian patients are at a higher risk of persistent hypopigmentation and should therefore not be treated as such. More rigorous studies, in particular RCT's comparing different treatment modalities, will generate stronger evidence.

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Chapter 6

A new argon gas-based device for the treatment of keloid scars with the use of intralesional cryotherapy

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Abstract

Background

Intralesional (IL) cryotherapy is a new promising technique for the treatment of keloid scars, in which the scar is frozen from inside. Multiple devices are available, mostly based on a simple liquid nitrogen Dewar system, which have a limited freezing capacity. Argon gas-based systems ensure accurate and highly controlled freezing and have shown to be effective within the field of oncologic surgery. However, this technique has never been used for treatment of keloid scars.

Objective

This prospective study evaluates an argon gas-based system for the treatment of keloids in a patient population including all Fitzpatrick skin types within a 1-year follow-up.

Methods

Twenty-five patients with 30 keloid scars were included and treated with a device called Seednet (Galil Medical, Yokneam, Israel). Scar quality and possible scar recurrence were assessed before treatment and post treatment (6 and 12 months) with objective devices determining scar colour, scar elasticity, scar volume and patient's skin type. In addition, scars were evaluated using the Patient and Observer Scar Assessment Scale.

Results

After 12 months, a significant volume reduction of 62% was obtained, p=0,05. Moreover, complaints of pain and itching were alleviated and scar quality had improved according to the Patient and Observer Scar Assessment Scale. Scar pigmentation recovered in 62% of all keloid scars within 12 months.

Five out of 30 (17%) scars recurred within 12 months, three of which had previously been treated with liquid nitrogen-based IL cryotherapy. Both recurrence and persistent hypopigmentation were mainly seen in Afro-American patients.

Conclusions

Intralesional cryotherapy with the use of an argon gas-based system proves to be effective in the treatment of keloid scars, yielding volume reduction and low recurrence rates. Although hypopigmentation recovered in most cases, it is strongly related to non-Caucasian patients. Finally, additional treatment of keloid scars previously unresponsive to IL cryotherapy is predisposed to a high recurrence rate.

Introduction

Keloids are tumour-like fibrous nodules, which result from an abnormal healing response of the injured skin. They can greatly reduce the quality of life by causing a cosmetic burden as well as physical complaints of pain and pruritis.[1–4] The treatment of keloid scars is a great challenge with high recurrence rates and even growth stimulus following treatment as the main problems.[1] Intralesional (IL) cryotherapy is a promising treatment technique in which the scar tissue is frozen from *within* the lesion. It was introduced by Weshahy in 1993 to overcome problems commonly associated with surface or external cryotherapy.[5] Since then, promising results have been released.[6–10]

Multiple devices are available for the use of IL cryotherapy, most based on a simple Dewar cylinder, in which liquid nitrogen is stored. The scar is frozen from inside by a hollow cryoneedle, which is attached to the Dewar cylinder through an elongation tube allowing liquid nitrogen to be passed through the needle.

Our experience in clinical practice has shown the freezing capacity of these devices to be limited, occasionally resulting in elongated freezing times and even in dysfunctional treatments. This not only is undesirable from a therapeutic point of view but even resulted in traumatic experiences for the patients, since in those cases, (local) anesthesia had already been administered.

Argon gas-based systems relying on the Joule-Thomson effect require no precooling (as opposed to the available liquid nitrogen devices) and offer controlled and accurate freezing.[11] Freezing is induced through the rapid expansion of argon gas through a small valve situated at the tip of the needle. Clinically, these systems are well integrated and showed excellent outcomes in prostate and renal oncology cryoablation surgery.[12–14] Therefore, we designed a prospective study to evaluate this promising technique for the treatment of keloid scars. Scar assessment was performed using the Patient and Observer Scar Assessment Scale and four objective devices to determine the patient's *skin type*, scar *colour*, scar *elasticity* and scar *volume*.[15,16]

Patients and methods

Patients

All patients visiting the plastic surgery department of the VU University Medical Center (Amsterdam, the Netherlands) between 2010 and 2012, meeting inclusion criteria, were included in a prospective study. Inclusion criteria were: 1) Patients with keloid scars.[1] Keloids were distinguished from hypertrophic scars based on the clinical judgment of their growth pattern and were defined as a fibroproliferative disorder of the skin that grew beyond the boundaries of the original wound or had an unrecognized origin, as described by Ogawa.[17] 2)

The period between previous treatment and IL cryotherapy covered a minimum of 6 months. 3) Patients with all Fitzpatrick skin types.[18] 4) Patients older than 10 years of age. Exclusion criteria were: pregnancy, diabetes mellitus and patients with collagen diseases.

Patient characteristics are listed in Table 1. Twenty-seven patients were included, of which two were lost to follow-up. Twenty-five patients with a total of 30 keloid scars completed the 1-year follow-up. Five patients with two scars were included, whose scars were of different etiology, scar duration, and anatomical location. These scars were therefore considered as separate entities.

Table 1. Patient characteristics

Scar	Age (yr)	Sex (m/f)	Duration (yr)	Location	Pre-treatment	Etiology	Fitzpatrick Score
1	10-20	F	6	Ear	ST	Surgery	5/6
2	20-30	F	8	Ear	Surgery	Surgery	5/6
3	40-50	M	5	Other	ST	Surgery	5/6
4	20-30	M	3	Face	ST	Infection	5/6
5	40-50	M	11	Ear	ST	Surgery	5/6
6	50-60	F	3	Other	St, ILC	Surgery	5/6
7	10-20	F	3	Ear	ILC	Surgery	5/6
8	10-20	F	3	Ear	Surgery, ILC	Surgery	5/6
9	50-60	M	16	Sternum	ILC	Trauma	5/6
10	50-60	M	16	Face	St, ILC	Trauma	5/6
11	30-40	F	8	Back	ILC	Surgery	5/6
12	50-60	F	-	Extremity	ST	Trauma	4
13	10-20	F	1	Ear	None	Surgery	4
14	30-40	F	3	Back	None	Trauma	4
15	10-20	M	3	Ear	ST	Surgery	4
16	30-40	M	4	Ear	ST	Surgery	4
17	50-60	F	-	Extremity	ST	Trauma	4
18	10-20	F	1	Ear	None	Surgery	4
19	30-40	F	3	Back	None	Trauma	4
20	50-60	F	6	Umbilicus	ST, ILC	Surgery	4
21	60-70	F	4	Sternum	ILC	Burn	3
22	20-30	F	2	Ear	ST, ILC	Trauma	3
23	60-70	F	6	Back	ST	Surgery	3
24	60-70	F	6	Sternum	ST	Surgery	3
25	20-30	M	6	Ear	Surgery	Surgery	3
26	80-90	M	6	Sternum	Surgery	Surgery	3
27	30-40	M	9	Sternum	ST	Other	3
28	60-70	F	3	Sternum	ST	Surgery	3
29	40-50	M	4	Back	ST	Infection	2
30	20-30	F	3	Sternum	None	Surgery	1

ST: intralesional steroid injection, ILC: intralesional cryotherapy

The study group consisted of 11 males and 14 females with a mean age 41.5 (range 17-84) and mean scar duration of 8 years (range 1-34). Mean scar size was 2.86 ± 0.5 cm^3 (range 0.2-8.8). Patients with all Fitzpatrick scores (F1-6) were included: 30% of the patients had a F1-3 skin type (Caucasian/Mediterranean) and 70% a F4-6 skin type (Asian/Afro-American). Most patients were previously treated with other scar treatments, of which all covered a minimum of 6 months until current treatment. Eight patients had previously been treated with liquid nitrogen-based IL cryotherapy, received an additional argon gas-based IL-cryotherapy 6 months later, due to unsatisfactory results. Patients were subdivided according to Fitzpatrick score into two groups: Fitzpatrick (F) 1-3; Caucasian/Mediterranean patients, F3-6; Asian patients/Afro-American patients.

Surgical procedure

Skin surface was disinfected and the scar was anesthetized locally using an extralesional approach with bupivacaine 0.5% with adrenaline. Next, the 17-gauge cryoneedle (IseSeed®, Galil Medical, Yokneam, Israel) was introduced longitudinally at mid-height and mid-width of the scar in a forward rotary movement until the center was reached. The needle was connected to the Seednet® device (Galil Medical, Yokneam, Israel), which uses high-pressured Argon (freezing) and Helium (warming) gases, supplied from an external gas source, via the same needle. Freezing and subsequent thawing intensity can be controlled in a percentage fashion to control the procedure. With larger keloids, multiple insertions were required to completely freeze the scar. The use of multiple needles simultaneously was also possible. After the scar was clinically completely frozen, the cryoneedle was gently removed with the help of the thaw function. Afterwards, sterile dressings were applied and patients were instructed to wash the treated scar daily with water.

Evaluation

All patients were consulted by both surgeon and researcher before treatment, gave informed consent and agreed with the follow-up. Independent researchers assessed the scars and patients' personal data and details on the origin of the scar were documented in a standardized Case Report Form. Patients were seen before treatment and post-treatment and then at 6 and 12 months post treatment for follow-up. Evaluation consisted of: 1) Comparison of photographs, taken before treatment and at each of the follow-up consultations. 2) Judgment of recurrence, defined as a growing, pruritic, nodular scar as described by Cosman and Wolff.[19] 3) Judgment of hypopigmentation; persistent hypopigmentation was defined as hypopigmentation of the skin in > 50% of the treated area after 12 months. 4) Scar elasticity, measured in two parameters: *extension* and *elasticity*

(Cutometer Skin Elasticity Meter 575, Courage and Khazaka Electronic GmbH, Cologne, Germany).[20] 5) Redness (*erythema*) and pigmentation (*melanin*), which were measured using the DermaSpectrometer (Cortex Technology, Hadshund, Denmark).[21] 6) Scar *volume*, obtained by creating a mold of the scar with dental putty (Cavex CA37, Alginate impression material, CAVEX Holland, the Netherlands). 7) Skintype, classified according to the Fitzpatrick Classification Scale.[18] 8) Subjective scar evaluation, performed by two experienced medical doctors and the patient using the POSAS. Each item of the POSAS was scored using a 10-step score, in which 10 reflected 'worst scar imaginable' and 1 indicated 'normal skin'.[15,16,22] The observational study was approved by the medical ethical council of VU University in the Netherlands.

Statistical analyses

Data was analyzed using SPSS version 20.0 (SPSS, Inc. Chicago, Ill). After being tested for normality, a paired *t* test was applied. Non-normally distributed data was analyzed by means of the Mann-Whitney test. The significance criterion for all tests was set a 0.05.

Odds ratios and correlations were tested according to a linear or bivariate regression.

Results

The scar quality as measured with four objective devices and the Patient and Observer Scar Assessment Scale, improved within 12 months following treatment (see figure 1).

Volume

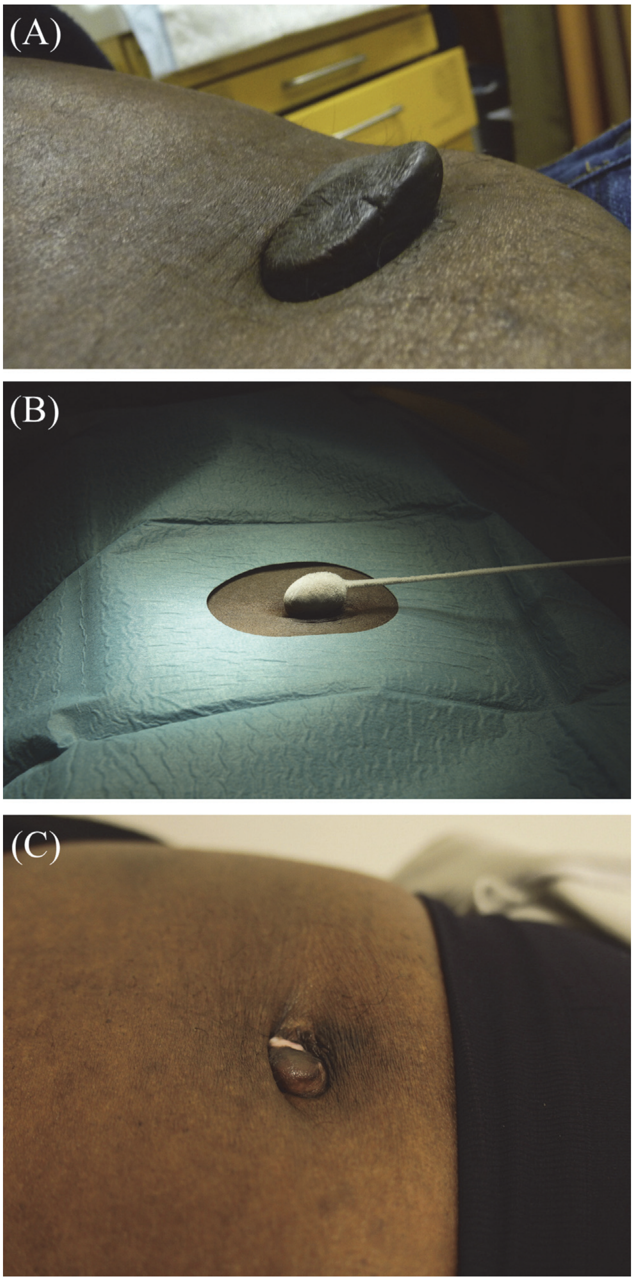
The average volume of the non-recurring keloid scars decreased from 3.4 ± 0.6 cm^3 before treatment to 1.3 ± 0.4 cm^3 12 months post treatment, indicating an average volume decrease of 62%, p=0.05. When analyzing volume decrease per Fitzpatrick skin type, F1-3 patients showed a 48% volume decrease after 12 months and F 4-6 patients a 66% volume decrease, p=0.026. Keloid scars, when previously treated with liquid nitrogen-based IL Cryotherapy showed an average 38% volume decrease after 12 months, p=0.028 (see figure 2).

Recurrence

Five out of 30 (17%) keloid scars showed a recurrence within 12 months post-treatment. The volume started to increase after 6 months (0 months (M): 2.2 ± 0.7 cm^3, 6M: 1.1 ± 0.4 cm^3, 12M 1.9 ± 0.5 cm^3).

Of those 5 patients, 4 were in the F4-6 group and 1 in the F1-3 group. The odds ratio for recurrence in F4-6 patients was 25 times higher compared to F1-3 patients. One has to take in account that 3 out of 5 recurring scars had previously been treated with nitrogen-based IL cryotherapy unsatisfactory.

Figure 1. Intralesional cryotherapy of a keloid scar located in the umbilicus, pretreatment (A), during treatment (B) and 12 months post treatment (C)



Redness

Scar redness, measured in erythema index, initially increased with 11% up to 6 months post treatment. After 12 months scar redness returned to pre-treatment values. (0M: 14 ± 1.7, 6M: 15 ± 1.9, 12M: 14 ± 3.2).

Figure 2. Volume decrease in cm^3 of non-recurring and recurring keloid scars after 12 months follow-up.

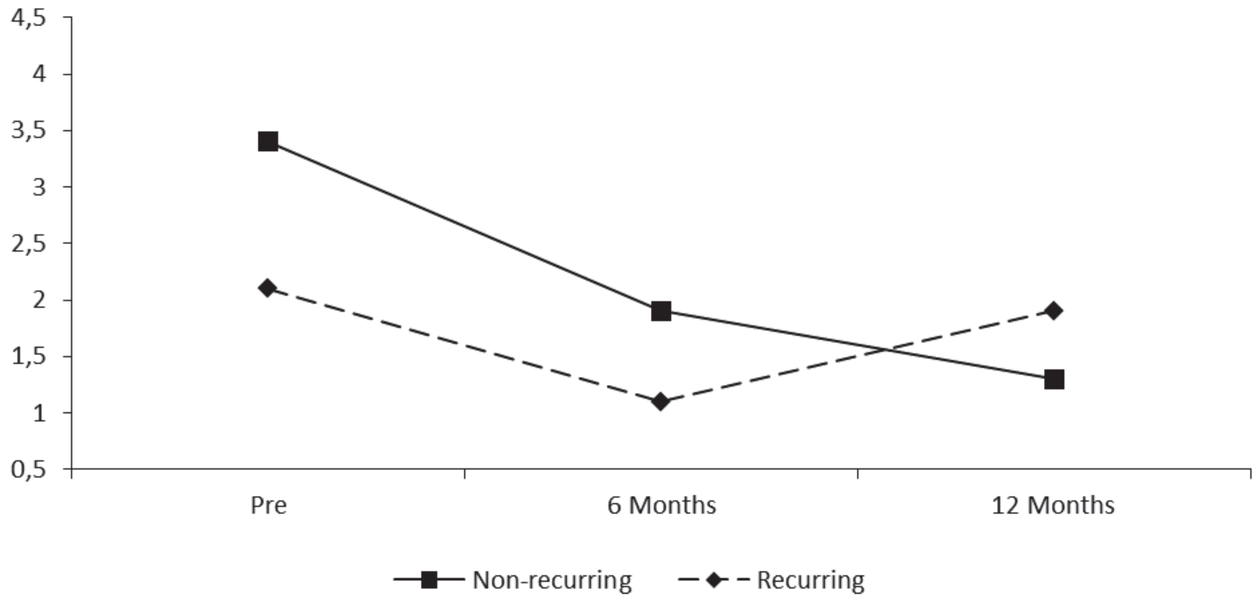
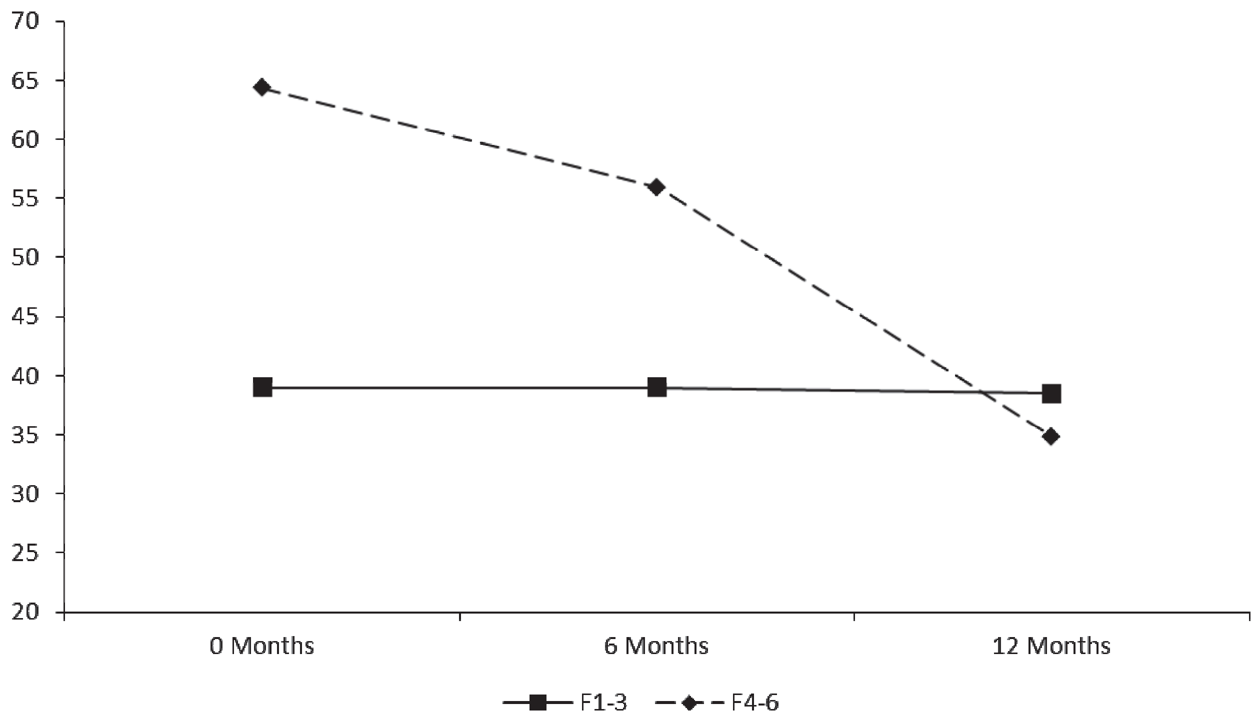


Figure 4. Skin pigmentation measured in melanin, divided in Fitzpatrick 1-3 skin type patients (Caucasian/Mediterranean) and F4-6 skin type patients (Asian/Afro-American).



Pigmentation

Although most scars showed partial or total loss of pigmentation after treatment, repigmentation was seen in 79% of the cases within 12 months. However, in 11 out of 30 keloid scars persistent hypopigmentation was observed after 12 months. See figure 3.

When analyzing these scars, it was noticed that 8 out of the 11 scars with persistent hypopigmentation, were of F4-6 patients. This was confirmed by the melanin index, which showed a pigmentation decrease in F4-6 keloid scars of 46%, in contrast to a 1% decrease in F1-3 scars after 12 months (see figure 4).

Elasticity

Scar elasticity was measured in millimeters and expressed the extent of skin stretching, reflecting skin thickness and rigidity.[20] Interestingly, while elasticity in F1-3 patients remained the same, elasticity in F4-6 patients improved with 36% after 12 months.

(F1-3 0M; 0.25 ± 0.1, 12M 0.24 ± 0.1, F4-6 0M; 0.48 ± 0.2, 12M 0.65 ± 0.3)

Patient and Observer Scar Assessment Scale

The Patient and Observer Scar Assessment Scale (POSAS), as assessed by the doctors and patients, showed an improvement after 12 months compared to the baseline score (the higher the score, the less the scar resembles normal skin).

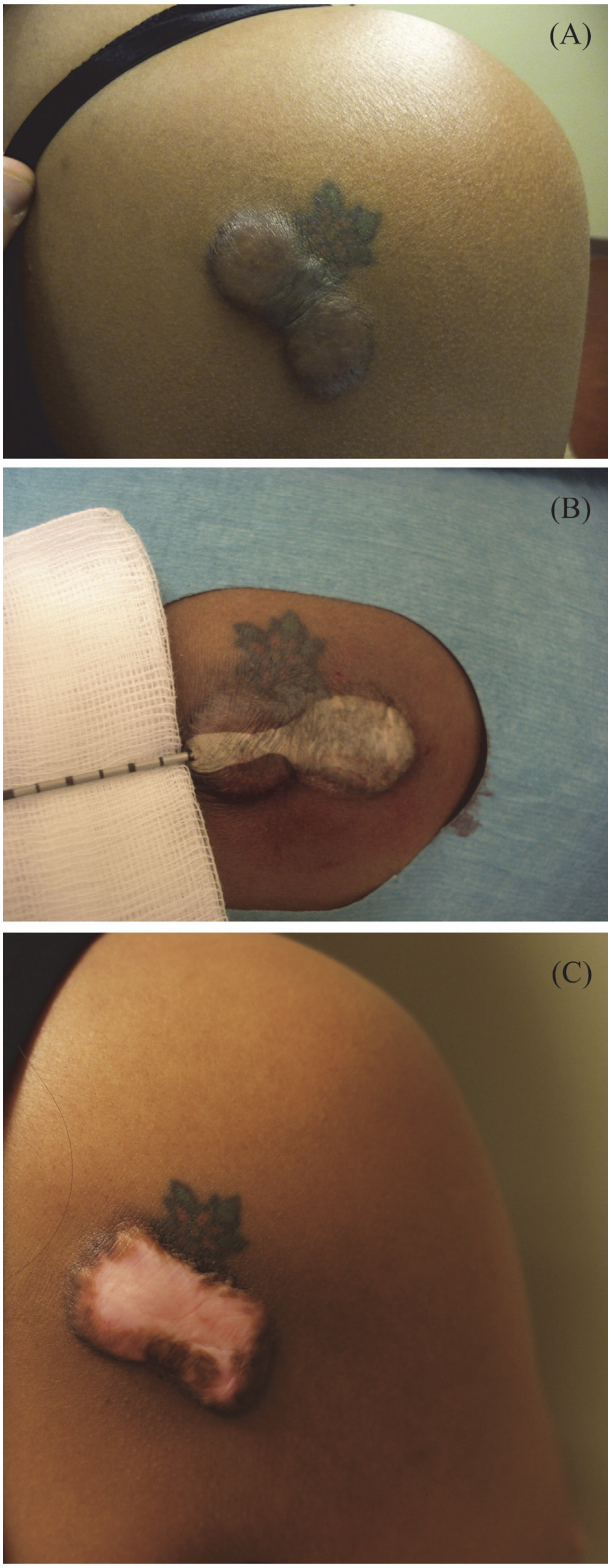
Patients scored a 32% improvement in the overall POSAS score (0M 37 ± 2.2, 12M 25 ± 3.9). In addition, patients scored 35% less pain and 33% less itching complaints after 12 months.[16]

In agreement to the patient's positive assessment, doctors scored an improvement of 9% in the overall POSAS score (0M 21 ± 0.9, 12M 19 ± 1.6). In contrast, keloid scars previously treated with IL Cryotherapy showed 13% deterioration in the overall score after 12 months as scored by the doctors (0M 22 ± 1.8, 12M 25 ± 4).

Complications

All patients reported mild to moderate postoperative pain, for which analgesics were prescribed in advance. Postoperative crusting and blistering were seen in all patients, which usually lasted for a maximum of 3 weeks. In 3 cases a wound dehiscence was seen, which were all treated conservatively.

Figure 3. Persistent hypopigmentation following 12 months of treatment with a keloid scar located on the back of a 25 year old female. Pretreatment (A), during (B) and 12 months post treatment (C).



Discussion

Intralesional (IL) cryotherapy has previously been proven effective for the treatment of keloid scars.[7,8,10] However, we found a limitation in the freezing capacity of the available liquid nitrogen-based devices. This led to the evaluation of an alternative novel device. Argon gas based closed systems have emerged within the field of renal and abdominal oncology because of their effective and safe functioning, but have never been described for the treatment of keloid scars.[23]

This prospective study presents the first results of IL cryotherapy with an argon gas based device for the treatment of keloid scars. Twenty-five patients with 30 keloid scars consisting of all Fitzpatrick skin types (F1-6) were treated and evaluated using four objective devices and the Patient and Observer Scar Assessment Scale to assess scar quality. Scar volume decreased significantly and complaints of pain and pruritus alleviated. Also, patients and doctors scored an improvement after 12 months with the Patient and Observer Scar Assessment Scale compared to before treatment.

Liquid nitrogen vs argon gas

In liquid nitrogen-based devices, a simple Dewar cylinder filled with liquid nitrogen is used as cryosource. When using this system repeatedly with multiple keloids, or large keloids, the freezing capacity is limited, leading to elongated freezing times and even in dysfunctional treatments. In addition, the freezing process cannot be closely controlled and monitored.

In contrast to liquid nitrogen-based systems, argon gas based systems work via pressurized gas expansion at the tip of an ultrathin cryoneedle. Per the Joule-Thomson effect, argon gas cools rapidly as it expands, creating the ice balls used for cryoablation only at the tip of the needle (as opposed to liquid nitrogen-based cryoneedles, which freezes along the entire track of the cryo-needle).[23] Conversely, the helium gas heats as it expands and is used to thaw the ice-ball formation rapidly. The freeze-thaw process is monitored precisely resulting in a controlled and accurate freezing process. Also, there is a possibility to monitor the internal tissue temperature by using thermocouples.

During treatment, we experienced a clear demarcation line between scar tissue and adjacent normal tissue (figure 3B). We hypothesize that the demarcation is due to the better vascularity of normal skin tissue compared to scar tissue. Hereby, cold is disposed quicker in normal skin tissue, resulting in a longer freezing process compared to scar tissue. This creates a demarcation between both tissues. Due to the demarcation line, IL cryotherapy is able to place the argon gas exclusively to the scar lesion, which is an advantage compared to other intralesional infiltration therapies.

Comparison with existing literature

This study shows an average keloid volume decrease of 62% with a 17% recurrence rate, 12 months after treatment. Patients reported a reduction in physical complaints of pain and itching. In another study performed by our group, we found a 61% volume decrease and a 20% recurrence rate for keloid scars following IL cryotherapy using an liquid nitrogen-based system.[6]

Other groups reported promising results in several studies using different open, liquid nitrogen based, devices in mostly small Caucasian patient populations.[7–10] However, the results of those studies were difficult to compare, because of the heterogeneity in study methods and outcomes of the published studies.[7–10] The mentioned studies included a relatively small Caucasian patient population and lacked a clear definition of recurrence and outcome measures. Also, they did not differentiate between keloid and hypertrophic scars and used different cryotreatment devices. Finally, follow-up was scored between 4-12 months.[7–10]

Persistent hypopigmentation

We observed persistent hypopigmentation in 11 out of 29 keloid scars, 12 months post-treatment, mainly in the F4-6 group (Asian/Afro-American patients). Other studies showed a high incidence of hypopigmentation for dark skinned patients as well.[9] This confirms that dark skinned individual are more prone to pigmentation problems.[24] The use of cryotherapy in non-Caucasian patients should therefore be avoided, since melanocytes are very sensitive to cold temperatures resulting in hypopigmentation.[25]

Unresponsive patients

This trial showed an excellent result of patients treated with argon gas based IL Cryotherapy. Only a little amount of the treated keloid scars showed recurrence (5 out of 30). Of these patients, 3 were treated previously with IL cryotherapy (liquid nitrogen-based) unsatisfactory. In our view, patients with an unsatisfactory result following IL cryotherapy need a different treatment of which excision followed by brachytherapy is a good option.[25]

The low recurrence rate may be explained by the end temperature induced by the argon gas based device (argon gas: -28°C). As Zouboulis describes, a tissue temperature of -20°C to -25°C is required to obtain a lethal temperature for the fibroblast, which is rather resistant to cold.[26–28] A lower end temperature would provoke lethal necrosis, which results in a less optimal scar quality and is therefore not essential for the treatment of benign skin lesions as keloid scars.

Limitations

Although this kind of research cannot be performed in a blinded setting, a randomized study including several scar treatments in a large patient population with all Fitzpatrick skin types would be favorable. Momently, our group contributes in such a study.[29]

Conclusion

Intralesional cryotherapy for treatment of keloid scars with the use of an argon gas based system shows promising results in terms of volume reduction and low recurrence rates. Moreover, argon gas based systems ensure an accurate and controlled freezing of scar tissue.

However, one has to take into account that patients having a dark skin are prone for hypopigmentation. Finally, we advise not to use this therapy in patients previously treated with IL cryotherapy unsatisfactory.

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Chapter 7

Comparison of two devices for the treatment of keloid scars with the use of Intralesional cryotherapy: an experimental study

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Wim Bloem
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Abstract

Background

Intralesional (IL) cryotherapy is a new technique for the treatment of keloid scars, in which the scar is frozen from *inside*. Two cryodevices are available, which were recently evaluated. Both devices showed promising results, but differed in clinical outcome. To explain these differences, more understanding of the working mechanism of both devices is required.

Objective

This experimental study was designed to investigate and compare the thermal behaviour of an *argon gas*- and a *liquid nitrogen*-based device. Thermal behaviour constitutes: 1) minimum tissue temperature (°C), 2) the freezing rate (°C/min). The thermal behaviour was measured inside and on the outer surface of the scar. Both devices were tested *ex vivo* and *in vivo.*

Results

Ex vivo, when determining the maximum freezing capacity, the argon gas device showed a higher end temperature compared to the liquid nitrogen device (argon gas: -120 °C, liquid nitrogen: -140 °C) and a faster freezing rate (argon gas: -1300 °C/min, liquid nitrogen: -145 °C/min).

In vivo, measured inside the keloid, the argon gas device showed a lower end temperature than the liquid nitrogen device (argon gas: -36.4 °C, liquid nitrogen: -8.1 °C) and a faster freezing rate (argon gas: -14.7 °C/min, liquid nitrogen: -5 °C/min). The outer surface of the scar reached temperatures below -20 °C with both devices as measured with the thermal camera.

Conclusion

In conclusion, the argon gas device displayed a lower end temperature and a faster freezing rate compared to the liquid nitrogen device. Although this resulted in lower recurrence rates for the argon gas device, more hypopigmentation was seen compared to the liquid nitrogen device following treatment. Finally, the low outer surface temperatures measured with both devices, suggest that some hypopigmentation following treatment is inevitable.

Introduction

Keloid scars result from an abnormal healing response after injury of the skin. Treatment is difficult, with high recurrence rates as the main problem. Cryotherapy is one of the treatment options.[1,2] For decades, liquid nitrogen has been applied externally by using a contact probe. However, to achieve cryonecrosis in the core of the keloid, a long hold time of the cryoprobe was required. Consequently, the surface epithelium was greatly affected, resulting in side effects such as blistering, hypopigmentation and infection.[1,2] To minimize epithelial damage, the hold time of the cryoprobe was limited, which resulted in higher recurrence rates and less volume decrease.[3]

To solve these problems, Weshahy introduced a new technique in 1993 called Intralesional (IL) cryotherapy.[4] With this technique, the scar is frozen from the inside; By using a hollow needle inserted in the scar, a cryogen is administered directly to the core of the keloid. In this manner, the exact location of the pathology is targeted, whilst the surface epithelium is less affected.[4] Two IL-cryotherapy devices are currently available; a liquid nitrogen-based device (Cryoshape) and an argon gas-based device (Seednet).[5,6]

In recent clinical studies, both devices showed a similar volume decrease (argon gas 62%, liquid nitrogen 63%). However, more hypopigmentation (argon gas 38%, liquid nitrogen 31%) and less recurrence (argon gas 17%, liquid nitrogen 24%) was reported using the argon gas device.[5,6] Importantly, the argon gas device study included 9 scars, previously treated unsatisfactory with liquid nitrogen-based IL cryotherapy. Out of these 9 scars, 3 developed a recurrence after treatment with the argon gas device. Excluding these patients would thus result in a recurrence rate of 9.5% for the argon gas device.

Two important parameters, the freezing rate and the minimum end temperature, constitute the *thermal behaviour* of these cryodevices. Differences in the thermal behaviour of both devices are likely to account for the dissimilar outcomes in both studies. To target fibroblasts inside the keloid low tissue temperatures (< -20 °C) are required. However, these temperatures are equally associated with surface complications such as hypopigmentation or wound dehiscence.[2] The freezing rate is of equal relevance as it determines ice-crystal formation. Fast freezing rates will cause cell necrosis, while slow freezing rates cause apoptosis, which has proven to be an irreversible process.[7]

To investigate and compare the thermal behaviour of a liquid nitrogen- and argon gas-based device, we designed an experimental study.

Methods and patients

The thermal behaviour of a liquid nitrogen-based (CryoShape®, Etgar Group International Ltd, Kfar Saba, Israel) and an argon gas-based cryoneedle (IseSeed®, Galil Medical, Yokneam, Israel) was measured. Both devices were investigated *ex vivo* to determine the maximum freezing capacity of the devices in an isolated test setting. Then, the devices were tested *in vivo* to evaluate the thermal behaviour in clinical practice.

Ex vivo test setting

A thermocouple (thermo measuring device) was attached by means of a strong wrapped narrow band of adhesive tape (3M Scotch Magic Tape) (clamp contact) to the distal end of the cryoneedle of the liquid nitrogen and the argon gas device. The temperature was recorded with a 4 Channel Pt100 Input Temperature Data Logger (Omega Engineering ltd. Toronto) and a data acquisition card (National Instruments NI PCI-6221). Data was processed using Labview and was summarized in the following outcome parameters: 1) minimum tip temperature of the needle (°C), 2) the freezing rate (°C/min). Prior to the measurements, the thermocouple was calibrated at three temperatures, melting ice (0 °C), boiling water (100 °C) and boiling liquid nitrogen (-195.8 °C). The thermocouple was mounted at the tip of the isolated needle, which was exposed to open air in a laboratory environment (20 °C). After the start of data acquisition, the needle was exposed to a cooling medium. The experiment was continued until the temperature of the tip of the needle reached a plateau. The freezing rate was calculated in the linear part of the graph, at the beginning of the test until the plateau phase.

In vivo test setting

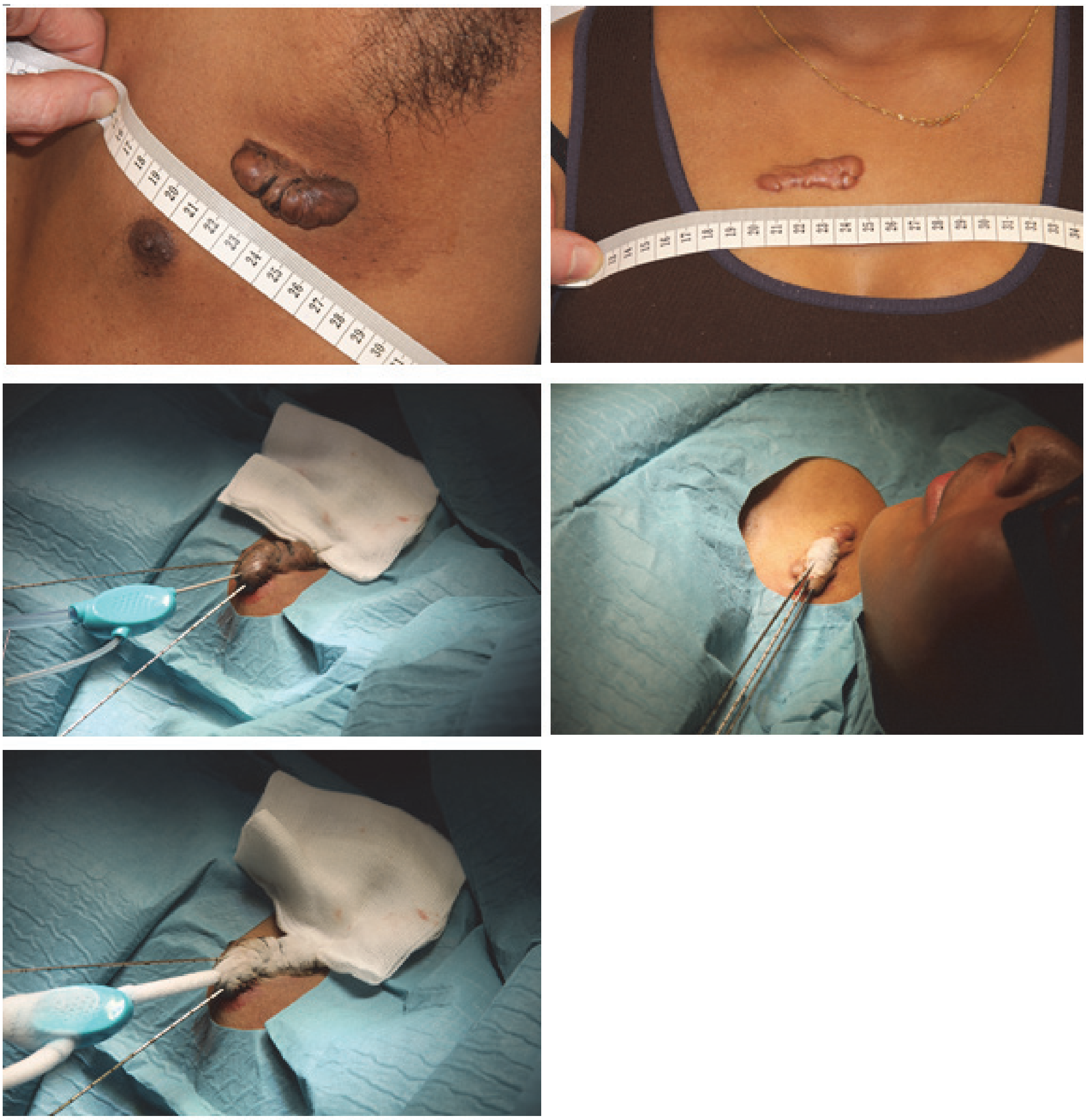
Patients

Eight patients with 10 keloid scars[12] were treated with IL cryotherapy at the plastic surgery department of the VU University Medical Center (Amsterdam, the Netherlands). Patients were randomly assigned to treatment with the *liquid nitrogen* or the *argon gas* device. Five patients with 6 keloid scars were treated with the liquid nitrogen device and 3 patients with 4 keloid scars with the argon gas device (table 1).

Procedure

The cryoneedle (liquid nitrogen device ór argon gas device) was introduced longitudinally at mid-height and mid-width of the scar in a forward rotary movement, as described in clinical studies.[9,10] Following the introduction of the cryoneedle, two thermocouples (thermo measuring device) were introduced to measure the internal temperature of the keloid scar (figure 1). One thermocouple was placed superficially (80% from the cryoneedle towards the surface) and one thermocouple deeper in the scar, at 20% distance from the cryoneedle (with a minimum of 4 mm).

Figure 1. In vivo test setting. The cryoneedle (argon-gas or liquid nitrogen-based) was introduced into the keloid scar. Then, two thermocouples (thermo measuring device) were introduced in a standardized manner: one thermocouple was placed superficially (80% from the cryoneedle towards the surface) and one thermocouple deeper in the scar at 20% distance from the cryoneedle. Intralesional cryotherapy with the liquid nitrogen-based device (pre-treatment: 1A, 1B, during treatment: 1C) and the argon gas-based device (pre-treatment: 1D and during treatment: 1E).



To measure the outer surface temperature, a thermal camera (Xenis Gobi 384) was positioned. The thermal camera did not record temperatures below -20 °C (253 Kelvin). See figure 3. The following outcome parameters were recorded with Labview: 1) minimum tissue temperature (°C). 2) freezing rate (temperature decrease per minute; °C/min) 3) scar surface temperature (°C). The medical ethical council of VU University in the Netherlands approved the study.

Table 1. Keloid size and characteristics

Keloid		Skin type (F)	Keloid location	Keloid size (cm) Length x width x height	Keloid aspect
Liquid nitrogen	**1**	3-4	Left shoulder	3 x 3 x 1.1	Erythematous, irregular boundaries
	2	2	Mid sternum	2.5 x1 x 1.2	Erythematous, smooth surface
	3*	5-6	Mid sternum	4.5 x 1.5 x 0.7	Stretched over surface sternum, irregularly shaped
	4*	5-6	Mid axilliary/nipple	5 x 1.5 x 2	Irregularly shaped protruding nodule
	5	3-4	Left shoulder	1 x 2.5 x 1.4	Erythematous, raised, protruding
	6	3	Suprasternal	2 x 3 x 1.5	Stretched, raised, erythematous
Argon gas	**1**	5-6	Mid Sternum	4.5 x 1.5 x 1.1	Hyperpigmented, irregularly boundaries
	2*	1	Left Shoulder	5 x 1.5 x 1.4	Erythematous, irregularly shaped, elevated
	3*	1	Right Shoulder	2 x 1 x 0.9	Erythematous, smooth surface
	4	5-6	Occipital	5 x 3 x 1.4	Irregular boundaries

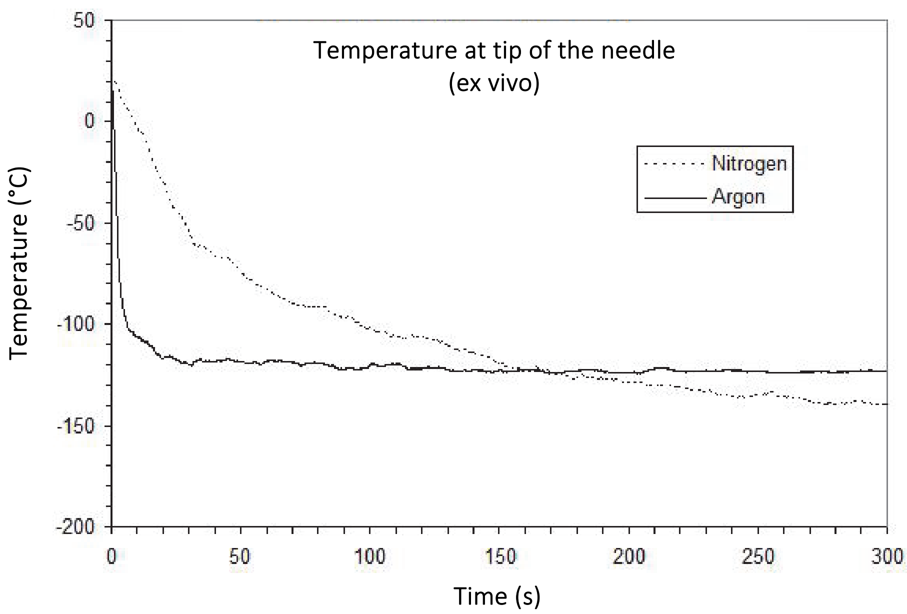
* same patient. Skin type: Fitzpatrick skin type (F) 1-2; Caucasian patients, F3-4; Mediterranean/Asian patients and F5-6; Afro-American patients.

Results

Ex vivo

When determining the optimal freezing capacity, the thermal behaviour of both devices differed substantially. The argon gas device showed a higher end temperature compared to the liquid nitrogen device (argon gas: -120 °C, liquid nitrogen: -140 °C) and a faster freezing rate (argon gas: -1300 °C/min, liquid nitrogen: -145 °C/min). See figure 2.

Figure 2. The figure shows the typical results of one of the ex vivo experiments with the liquid nitrogen-based device and the argon gas-based device: the argon-gas device displayed a faster freezing rate, but a higher end temperature compared to the liquid nitrogen device.



In vivo

In clinical practice, the thermal behaviour measured *in vivo* differed from the *ex vivo* results. See table 2.

Table 2. In vivo test results. Thermal behaviour of the liquid nitrogen-based and the argon gas-based device

		Internal temperature			
		Minimum temperature (°C)		**Freezing rate (°C/min)**	
		Superficial	**Deep**	**Superficial**	**Deep**
Liquid	1	-12.3	- 4.0	- 5.3	- 4.8
Nitrogen	2	-26.0	- 5.4	-14.1	- 8.1
	3*	-18.9	-25.7	-12.8	-14.6
	4*	- 7.0	- 5.4	- 1.6	- 1.4
	5	- 9.0	- 6.0	- 4.8	- 4,1
	6	- 1.0	- 2.0	- 5.2	- 6.7
	Average	**-12.4± 8.9**	**- 8.1±8.7**	**- 7.3±4.9**	**- 6.6±4.5**
Argon	1	-39.7	-31.5	-26.1	-23.3
gas	2*	-50.0	-28.5	-13.3	- 9.4
	3*	-33.0	-32.0	- 9.2	- 9.1
	4	-23.0	-18.0	-10.0	-11.7
	Average	**-36.4±11.4**	**-27.5±6.5**	**-14.6±7.8**	**-13.4±6.7**

* same patient.

Internal temperature

The superficial thermocouple measured, on average, lower temperatures with the argon gas device compared to the liquid nitrogen device (argon gas: -36.4 °C, range: -50 °C to -23 °C. Liquid nitrogen: -12.4 °C, range: -26 °C to -1 °C).

The deep thermocouple displayed the same trend as the superficial thermocouple, although less cold temperatures were reached (argon gas: -27.5 °C, range: -18 to -32 °C. Liquid nitrogen: -8.1 °C, range: -2 to -25.7 °C). Notably, the liquid nitrogen device showed no consistency in the reached temperature since a large range was measured, both superficially and deeply.

Internal freezing rate

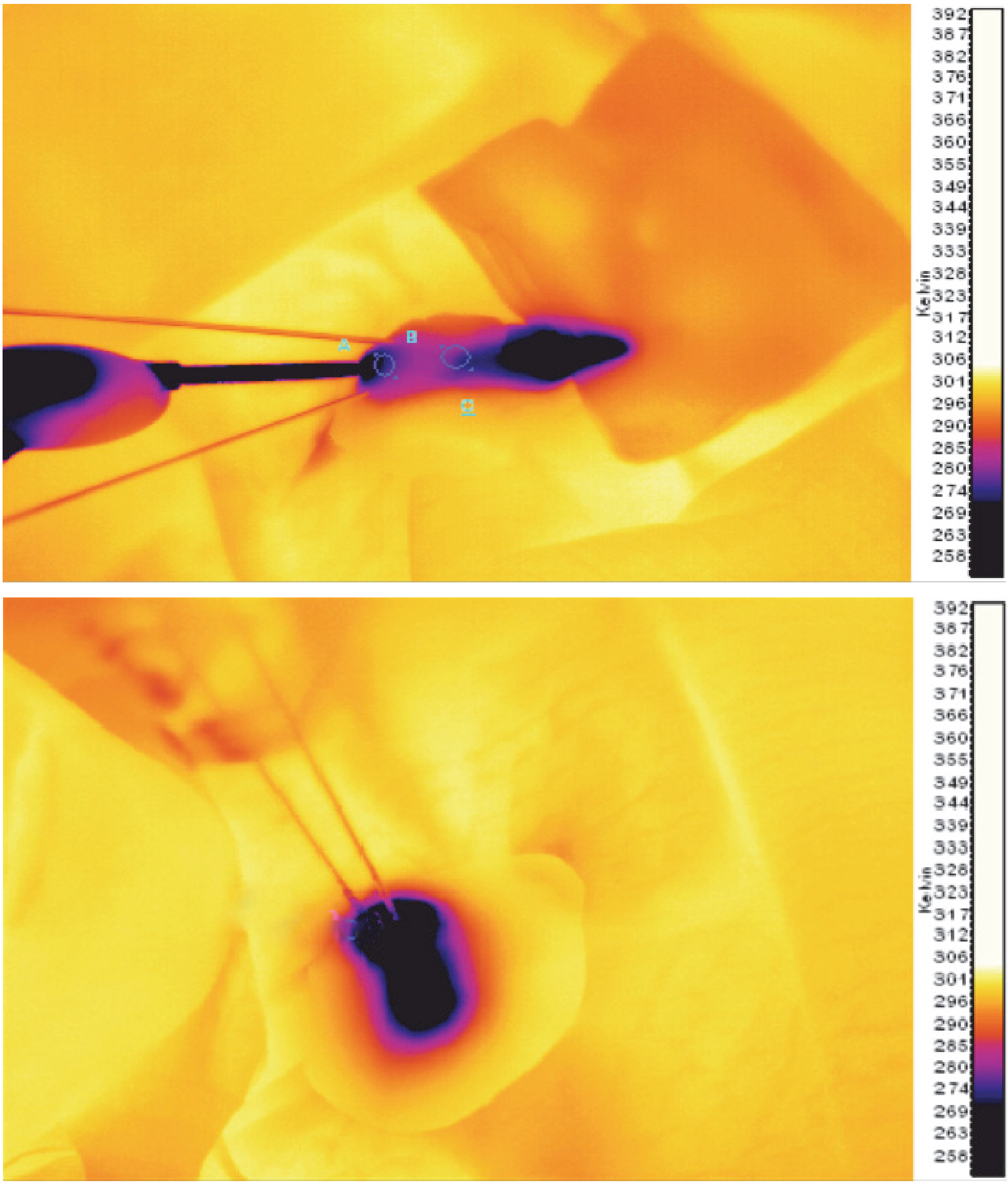
The superficial thermocouple measured higher freezing rates with the argon gas device compared to the liquid nitrogen device (argon gas: -14.7 °C/min, liquid nitrogen: -7.3 °C/min). The deep thermocouple measured the same trend as the superficial thermocouple (argon gas: -13.4 °C/min, liquid nitrogen: -5.0 °C/min).

Outer surface temperature

The thermal camera measured the outer surface of the keloid scars during treatment.

Figure 3 shows a thermal camera image during treatment with the liquid nitrogen device (a) and with the argon gas device (b). During treatment with the argon gas device, temperatures below -20 °C were measured on the entire surface of the skin of the lesions. Treatment with the liquid nitrogen based device induced equally low temperatures, however these temperatures were only measured at the site of needle insertion and the middle of the lesion.

Figure 3. **Outer surface temperature as measured with the thermal camera during treatment with the liquid nitrogen-based device (3A) and the argon gas-based device (3B). The darker the colour, resembles a lower temperature. The thermal camera did not record temperatures below -20 °C (253 Kelvin).**



Discussion

Intralesional (IL) cryotherapy is a novel technique for the treatment of keloid scars. Two cryodevices are currently available, a liquid nitrogen-based and an argon gas-based device.

Clinical studies, evaluating both devices, displayed a higher hypopigmentation rate, but a lower recurrence ratio after treatment with the argon gas device compared to the liquid nitrogen device. To explain these differences, more information was needed about the working mechanism of both devices and the tissue reached temperatures during treatment. Therefore, we investigated the thermal behaviour (freezing rate and minimum end temperature) of both devices *ex vivo* and *in vivo*.

Ex vivo vs in vivo experiments

Differences in outcomes between the *ex vivo* and *in vivo* experiments were seen in this study. During the *ex vivo* experiments, the tips of both cryoneedles were situated in air which causes a relatively small heat flow from the environment to the needles. It was therefore nearly an insulated condition in which the devices were tested. In this situation, the liquid nitrogen device reached a lower end temperature than the argon gas device (argon gas: -120 °C, liquid nitrogen: -140 °C). This is explained by the lower boiling temperature of liquid nitrogen compared to argon gas.[7]

In contrast, during the *in vivo* experiments, the argon gas device displayed lower end temperatures than the liquid nitrogen device (superficial thermocouple; argon gas: -36.4 °C, liquid nitrogen:-12.4 °C. Deep thermocouple: argon gas: -27.5 °C, liquid nitrogen: -8.1 °C). This difference of the end temperature between the *ex vivo* and *in vivo* experiments may be explained by the basic physical principles of the cooling method of both devices. With the liquid nitrogen device, the needle is cooled by a forced flow of liquid nitrogen by pressing the liquid nitrogen out of a Dewar vessel. The liquid nitrogen has to pass a conduit (tube) from plastic and stainless steel before it enters the needle. Since all these tubes were not insulated, the liquid nitrogen device is mostly uninsulated and thereby loses its freezing capacity before entering the keloid scar. In contrast, with the argon gas device, the tip of the needle is cooled directly due to the Joule-Thomson cooling process which occurs within the tip of the needle.[10] On the other hand, *in vivo*, the human body is heated actively by the blood circulation which demands a higher freezing capacity.

Thus, the uninsulated liquid nitrogen device and the demanding freezing capacity of the human body led to a reduced freezing capacity for the liquid nitrogen device with the *in vivo* experiment compared to the *ex vivo* experiments resulting in higher end temperatures. In contrast, no freezing capacity is lost with the argon gas device, using the Joule-Thomson process for cooling in the tip of the needle, resulting in a lower end temperature compared to the liquid nitrogen device during the *in vivo* experiment.

Minimum end temperature

For the treatment of benign skin lesions, a tissue temperature of -20 °C to -25 °C is preferred.[2] In this study, the argon gas device displayed temperatures below -25 °C measured inside the scar (superficial: -36.4 °C, deep; -27.5 °C). Although these low temperatures will be effective for cell destruction, it may have led to unnecessary tissue damaging. Opposed, the liquid nitrogen reached fairly higher end temperatures (superficial: -12.4 °C, deep; -8.1 °C). These warmer tissue temperatures won't be lethal to fibroblasts, which are relatively resistant to cold, resulting in more recurrences.[2] Besides the *internal* tissue temperature (superficial and deep thermocouple), the outer *surface* temperature (thermo camera) was also measured in this study. The surface temperature is relevant, because melanocytes, located in the surface epithelium, are very sensitive to low temperatures and die at -4 to -7 °C.[2,11] When temperatures reach below -20 °C to -30 °C, no repigmentation occurs.[11]

Using a single thermocouple, Har-Shai measured an outer surface temperature of -15.5 °C during IL cryotherapy with a liquid nitrogen device.[5] In order to analyze the complete scar surface, we used a thermal camera and found surface temperatures below -20 °C using the same liquid nitrogen device. However, these low temperatures were limited to the center of the lesion and the insertion point of the cryoneedle. In contrast, with the argon gas device, temperatures below -20 °C were measured on the entire surface of the skin of the lesions. These more extended low surface temperatures can be the explanation for the higher persistent hypopigmentation rate seen with the argon gas device compared to the liquid nitrogen device.[5,6] More importantly, the data indicates that some degree of melanocyte destruction is inevitable with the use of IL cryotherapy.

Freezing rate

This study displayed a faster freezing rate for the argon gas device compared to the liquid nitrogen device. This is an important finding, since the freezing rate is one of the main factors in cell destruction.[7] Cell destruction is caused by ice crystal formation.[7] Ice-crystal formation is dependent on the freezing rate (°C/min), which can be divided in slow, fast and ultrafast. A slow freezing rate, <25 °C/min, will result in the formation of extracellular ice only, as the intracellular space is being protected by the highly insulating cell membrane. Eventually, the cell will be dehydrated through osmosis and will die through apoptosis. In contrast, a high freezing rate (>25 °C/min) results in both extra- and intracellular ice crystal formation.[13] The intracellular ice crystals act like blades destructing the cell membrane, leading to necrosis.[13]

Whereas oncologic treatment requires a fast freezing rate to achieve lethal necrosis, this is not necessary for benign skin lesions.[2]

The argon gas device, designed for oncologic surgery,[14-16] displayed higher freezing rates compared to the liquid nitrogen device in this study. The higher freezing rate may have resulted in more necrosis in the core of the keloid, resulting in a low recurrence ratio. However, it may have also attributed to the higher hypopigmentation and wound dehiscence ratio seen following treatment with the argon gas device compared to the liquid nitrogen device.[5,6]

Conclusion

In conclusion, the thermal behaviour of the argon gas- and liquid nitrogen-based devices differed both ex vivo and in vivo. The argon gas device displayed a lower end temperature and a faster freezing rate compared to the liquid nitrogen device. Although this resulted in lower recurrence rates for the argon device, more hypopigmentation was seen compared to the liquid nitrogen device following treatment. Finally, the low outer surface temperatures measured with both devices, suggest that some hypopigmentation following treatment is inevitable.

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Part III
Excision with adjuvant irradiation for treatment of keloid scars

Chapter 8

Surgical excision with adjuvant irradiation for treatment of keloid scars; a systematic review

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Abstract

Introduction

Excision followed by adjuvant irradiation is considered safe and most efficacious for treatment of keloid scars. Recently, different authors published successful treatment protocols and recommended the following issues: 1) The use of *high-dose-rate (HDR) brachytherapy* instead of *low-dose-rate (LDR) brachytherapy* or *external radiation*. 2) A short time interval between operation and irradiation. 3) Single-fraction instead of multi-fraction irradiation. 4) A minimum of 12-24 months follow-up post-treatment.

Methods

This study evaluates the above recommendations with a systematic review of the English-language literature, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. Both PubMed and Embase were searched. Studies were graded according to the American Society of Plastic Surgeons Rating Levels of Evidence.

Results

Thirty-three studies were selected. Six studies were graded as Level of Evidence type II studies and 27 as type III. HDR brachytherapy showed lower recurrence rates compared to LDR brachytherapy and external radiation. A short time (<7 hours) interval between scar excision and irradiation results in a lower recurrence rate compared to longer time-intervals (>24 hours). Single-fraction irradiation showed promising results in terms of recurrence rate and patient convenience. Finally, scar recurrences were seen between 2 and 36 months, with a mean of 15 months.

Conclusions

Based on this systematic review of the literature, the evidence confirms the recommendations stated by authors in the recent years. However, due to the lack of high-quality randomized studies, the quality of this evidence is limited. More randomized studies will generate stronger recommendations.

Introduction

Keloid scars are a benign fibroproliferative disease impairing the quality of life of patients by causing cosmetic disfigurement and complaints of pain and pruritus.[1,2] Treatment is difficult with high recurrence rates and even growth stimulus as the main issue.[1] According to the international advisory panel on scar management, surgical excision with post-operative radiation therapy is considered the most efficacious treatment.[3]

Radiation therapy for treatment of keloid scars was first described by Sequira in 1909.[4] Traditionally, it was applied *externally* by a variety of devices.[5] Although good results were achieved, external radiation therapy requires a relatively high irradiation dose due to the large distance between the radiation source and the scar. Also, the surrounding healthy skin is unnecessarily exposed to radiation.[6]

To solve these problems, Malaker et al. introduced a technique called *brachytherapy* (also called *interstitial* or *internal radiation)* in 1976.[6] Nowadays, it is available as low-dose-rate (LDR) or high-dose-rate (HDR) brachytherapy. In both methods, a hollow catheter is incorporated in the surgical lesion after excision of the scar, through which a radioactive source is directed. In this way, irradiation is effectively localized from inside the lesion, only targeting the desired area.[6] With LDR brachytherapy, a low dose radioactive source is used and removed after typically 20-72 hours.[7] In contrast, with HDR brachytherapy, a high radioactive source is applied for a short period of 5-10 minutes.[8] Due to the short treatment time, HDR brachytherapy is an out-patient procedure enhancing patient convenience, whereas LDR brachytherapy requires hospitalization (see figure 1).

Recently, different authors described new protocols aiming to reduce keloid recurrence and improve patient convenience.[7-14] They recommended the following issues: 1) The use of HDR brachytherapy instead of LDR brachytherapy or external radiation.[9,8] 2) A short time interval between operation and irradiation.[7,8] 3) Single-fraction instead of multi-fraction irradiation.[11–14] 4) A minimum of a 12-24 months follow-up post-treatment.[15,16] This systematic review evaluates these recommendations.

Methods

Search strategy

A comprehensive systematic review of the English-language literature was performed, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. PubMed and Embase were searched from inception to 14 January 2014 and 23 January 2014, respectively. The following terms were used as index terms or free-text words: ‘cicatrix’ or ‘scars’ (including synonyms and closely related words as hypertrophic scar and keloid scar) and 'brachytherapy' or 'x-ray therapy' or 'surface radiotherapy'. The full search strategies for PubMed and Embase can be found in the Supplementary Information.

Figure 1. Surgical excision with adjuvant brachytherapy. Example of an auricular keloid scar (1a) with surgical excision (1b). Then a catheter is positioned between the dermal edges of the wound, below the surface of the skin and extending out of the skin beyond the wound (1c). Postoperatively, the patient will be transferred to the radiation department for the adjuvant High-Dose-Rate Brachytherapy (source: Van Leeuwen et al.[8])

References of retrieved articles were scanned for additional studies. Inclusion criteria consisted of the following: 1) Any English-language randomized controlled trials (RCTs), controlled clinical trials (CCTs) or prospective or retrospective cohort studies reporting surgical excision (primary closure, no use of skin grafts) with adjuvant radiotherapy for treatment of keloid scars; 2) A minimum follow-up duration of one year for all lesions 3) Studies including solely keloid scars or studies with a clear definitions distinguishing hypertrophic and keloid scars and separate analysis for both lesions; 4) No adjuvant interventions following surgical excision other than radiation therapy; 5) Studies measuring recurrence rate as outcome, based on the regrowth of the keloid scars with or without functional complaints[8] 6) Poster abstracts, case reports or letters to the editor were not included. In case of duplicate articles only one was included.

The article screening process was performed as follows: Three investigators (ML, SS and JK) carried out the initial searches and two investigators (ML and SS) independently reviewed the studies for eligibility. Investigators were blinded to each other, meeting only to compare findings after completing the extraction process. Decisions about eligibility were resolved by discussion. Seventy potentially relevant studies were identified from the initial searches. Subsequently, 2 authors (ML and SS) independently screened the full-text articles for eligibility using a standardized data abstraction form with inclusion and exclusion criteria. Disagreement was resolved by discussion. This eventually resulted in 33 articles. See figure 2.

Data extraction

One reviewer extracted data and a second review author verified the accuracy of the extracted data. Discrepancies in opinion about an article were reviewed and consensus was achieved through discussion. A standardized data form was used to obtain the following information:

1) Study characteristics; 2) Study participants (including origin or Fitzpatrick score); 3) Study design (prospective/retrospective, follow-up duration; 4) Intervention, including type of radiation. Type of radiation was divided into external radiation (all different external devices including the surface applicator), LDR brachytherapy and HDR brachytherapy. Also, radiation dosage and radiation scheme were extracted; 5) Study results, of which the recurrence rate was the main outcome. Thereafter, data were arranged in evidence tables according to type of radiation.

Methodological quality assessment

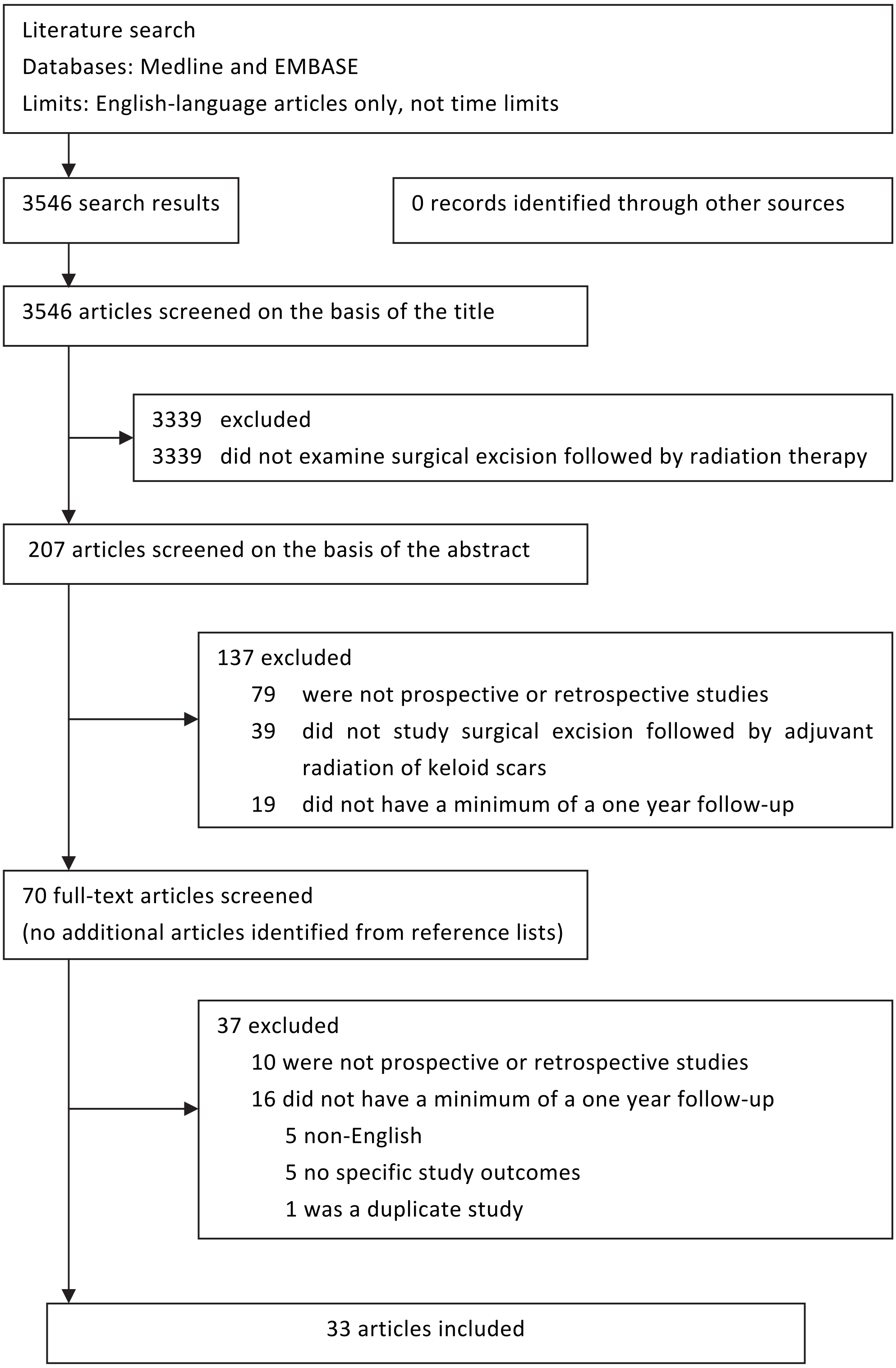
Heterogeneity in study design and outcome measures, did not allow for quantitative pooling of data for meta-analysis. The extracted studies were graded according to the American Society of Plastic Surgeons Rating Levels of Evidence.[17] This classification assigns each article to a corresponding level of evidence ranging from I (highest) to V (lowest). We classified a level II study to prospective studies, with a clear definition of keloid scars[18] and recurrence.[19]

Results

Study characteristics

Initial database searches identified 3546 articles. A flow diagram of the search and selection process is shown in figure 2; 3339 articles were eliminated based on the title of the manuscript, because there was no relation between radiation therapy and keloid scars. Next, 207 abstracts were screened, of which 137 were excluded for not meeting with the selection criteria. Thus, 70 full-text articles were analyzed. Sixteen studies were excluded because they did not respect a minimum of a one year follow-up, 10 studies were not prospective or retrospective, 5 were not in English, 5 had no specific outcome measures and there was one duplicate study. Finally, 33 articles met all inclusion criteria. See figure 2.

Figure 2. Flow diagram of the search and selection process according to PRISMA



A summary of the characteristics of the included studies is given in table 1. Of the included articles, 10 were from the United States, 4 from Japan and 4 from India. The remaining 15 studies were from 9 different countries. Twenty-five studies used external radiation, 5 used HDR brachytherapy and 3 LDR brachytherapy. The mean publication date was most recent for studies using HDR brachytherapy (HDR: 2005; range 2001-2008, external: 1996; range: 1970–2013, LDR: 1992; range: 1976–2009).

Methodological quality

We classified 6 studies with level of evidence type II and 27 studies with level of evidence type III. There were 2 RCT studies, randomizing different keloid treatment options, of which radiation was one. Twelve studies were prospective and 19 retrospective. See table 1.

Patient characteristics

The sample size of the included studies ranged from 12 to 501 patients (mean: 97.8 ± 18.8) with a total of 17 to 570 scars (mean: 111.9 ± 22.4). In total, 3130 patients with 3470 keloid scars were treated. The follow-up ranged from 12 to 239 months (mean 49 ± 9.5 months). Although all included studies mentioned a minimum of a one year follow-up, 42% did not describe the range of the follow-up completely. Patients' origin was noted in 57.6% of the studies: the majority treated a mixed population (74%), although origin or skin type was not always specified. Others treated solely Caucasian (5%), Asian (10.5%) or Afro-American (10.5% patients). The location of the keloid scars was mixed in 67% of the studies. In 18% of the studies, only keloid scars located on the earlobes were treated and 15% of the studies did not specify scar location. The mean age of the patients was 28.7 ± 1.3 years (range 2-82 years). In 35% of the studies age was not described.

Table 1. Summary of the included studies

Study	Study Type	N (P/L)	L	Fitzpatrick	Follow-up Mean (range)	Radiation (frac/dose)
External radiotherapy						
Ogawa et al., 2013	R	145/174	Ear	N/A	18M (N/A)	2/ 10Gy 2/ 15Gy
Kim and Lee, 2012	P	26/26	Abd	N/A	27 (19-36)	3/ 12-15Gy
Emad et al., 2010	P	26/76	Var	N/A	19 (12-24)	3/ 12Gy
Sakamoto et al., 2009	R	119/194	Var	F3-4; Asian	36 (12-164)	8/ 16-40Gy
Kar et al., 2007	R	21/32	Var	F1-6	19 (12-35)	3-4/ 12Gy
Akita et al., 2007	R	32/38	Var	N/A	50 (12-108)	4-11/ 12Gy
Ragoowansi et al., 2003	R	80/80	Var	F1-6	N/A (12-60)	1/ 10Gy
Ogawa et al., 2003	R	129/147	Var	N/A	24 (18-128)	3/ 15Gy
Maarouf et al., 2002	R	36/50	Var	N/A	84 (36-126)	3-5/9-15Gy
Perez et al., 2001	R	110/163	Var	F1-6	81 (24-239)	3/ 12Gy
Ragoowansi et al., 2001	P	35/35	Ear	F1-6	N/A (12-60)	1/ 10Gy
Wagner et al., 2000	R	139/166	Var	N/A	240 (1-N/A)	14Gy range: 7.5-28.5 Gy
Sclafani et al., 1996	RCT	42/50	Ear	F1-6	18 (12-N/A)	I: 1/ 10Gy II: 1/ 7Gy
Norris, 1995	R	24/24	N/A	F5-6	24 (N/A)	3/1200rads
Duronsinmi-Etti et al., 1994	P	244/454	Var	F5-6	24 (N/A)	1-3/5-15Gy
Chaudry et al., 1994	R	36/36	Ear	F3-6	67 (24-130)	3/ 18Gy
Darzi et al., 1992	RCT	100/58	N/A	N/A	24 (N/A)	I: Pre+post: 16 Gy II: Post: 2/16 Gy
Supe et al., 1991	P	64/64	Var	F3-4; Indian	12 (N/A)	4/ 20Gy
Doornbos et al., 1990	R	200/278	N/A	N/A	N/A (12-24)	2-4/ 15Gy
Kovalic and Perez, 1989	R	76/76	Ear	F-1-6	117 (13-239)	3/ 12Gy
Sällström et al., 1989	P	124/124	Var	F1-6	24 (N/A)	3/1800rads
Deka et al., 1987	P	86/86	Var	N/A	12 (N/A)	4/ 20Gy
Ollstein et al., 1981	P	40/86	Var	F1-6	24 (12- N/A)	3/1500rads
Inalsingh, 1974	R	501/NA	Var	F1-6	24 (N/A)	4Gy monthly
King, 1970	R	32/32	Var	F1-6	180 (N/A)	1-2/1000- 3000rads

Ref	< 24 hrs	Histol. Confir	Def R/K	Outcome measures	Recurrence	L/E
4Mev	no	no	-/-	Advise to treat earlobes with 10Gy divided over 2 fractions	10Gy: 4.6 % 15Gy: 4.9 %	**III**
6Mev	yes	no	-/-	96% of patients satisfied	23 %	**III**
120Kv	no	no	+/+	Complaints of pain and itching improved in all lesions	18.2 %	**II**
55-100KvP	no	no	+/-	Advice for 20Gy in 5 fractions	33 %	**III**
250Kv	yes	yes	+/+	48% judges their keloid recurrence as worse than pre-treatment	71.9 %	**III**
9Mev	no	no	-/-	- VSS: improvement - Durometer: softer scars	21 %	**III**
60Kv	yes	no	+/+	Early, single, postoperative radiation is simple and effective	16 %	**III**
4MeV	no	no	+/-	Significant more recurrence at high tension locations	32.7 %	**III**
5-6Mev	no	no	-/-	83% of the patients was very satisfied	16 %	**III**
4Mv	yes	no	-/-	Advice minimum of 2 year follow-up	33 %	**III**
100Kv	yes	no	+/-	Advice minimum of 2-3 year follow-up	20.60 %	**III**
N/A	no	no	-/-	A low dose of 8-10Gy may be sufficient	Mean: 20 % (range 8-33)	**III**
100Kv	yes	no	+/+	Radiotherapy appears more effective than steroid inject.	I: 12.5 % II: 0 %	**II**
100Kv	yes	yes	-/+	Transitory hyperpigmentation	53 %	**III**
50Kv	no	no	-/-	A short course for post-operative radiotherapy benefits the patient	7 %	**III**
100KV	yes	yes	-/+	Satisfactory results in 97.2%	2.8 %	**III**
N/A	no	yes	-/+	Failure rate: early (19%) vs late post-surgery radiation (43%)	I: 34 % II: 27 %	**III**
N/A	no	no	-/-	Fraction given weekly and biweekly	Weekly: 20 % Biweekly:35%	**III**
120Kv	no	no	-/-	Irradiation of a regrowing lesion following excision prevents recurrence	14.8 %	**III**
4Mv	yes	no	-/-	Size: ≤ 2cm: 85% success ≥ 2 cm: 47% success	27 %	**III**
50Kw	yes	yes	+/+	Slight hyperpigmentation in 31% of the patients	8 %	**II**
16.65 GBq	no	no	-/-	5Gy given biweekly most appropiate	30 %	**III**
100 KVP	yes	yes	-/+	-No difference Caucasian and non-caucasian. -If skin graft was needed -> pre-operation radiation	21 %	**III**
60-90KVP	no	no	-/-	Patients were treated on a monthly base, starting the 18^{th} day post-surgery	23.5 %	**III**
1-3 MV	no	no	-/-	Symptomatic relief in 52.6%	25.8 %	**III**

Study	Study Type	N (P/L)	L	Fitzpatrick	Follow-up Mean (range)	Radiation (frac/dose)
High-Dose-Rate Brachytherapy						
van Leeuwen et al., 2014	P	43/67	Var	F1-6	33 (24-96)	2/ 12Gy
Arneja et al., 2008	R	25/25	Ear	F1-6	36 (12-60)	3/ 15Gy
Veen and Kal, 2007	P	35/54	Var	N/A	12 (N/A)	I: 4Gy + 2x3Gy II: 6Gy + 2x4Gy III: 3x 6Gy
Garg et al., 2004	R	12/17	Var	N/A	26 (12-71)	3/ 15Gy
Guix et al., 2001	P	169/169	Var	F1-2	37 (13-85)	4/ 12Gy
Low-Dose-Rate Brachytherapy						
Arnault et al., 2009	R	31/55	Var	F1-6	84 (24-192)	17.9 Gy at 5mm
Escarmant et al., 1993	R	361/570	Var	N/A	82 (15-156)	20 Gy at 5mm
Malaker et al., 1976	P	30/31	Var	N/A	24 (N/A)	2000 rad at 2,5mm

NS: not specified in study, Type of study: P: prospective, R: retrospective, Pi: pilot study. N (P/l): Number of patients and lesions (patients/lesions). L: location of scar. Fitzpatrick skin type; ranging from 1-6. Radiation (fraction/dosagein Gray (GY)).

Excision and radiation type

Most studies used an extralesional approach to excise the scar (n=12), only one study[20] excised the scar intralesionally. Other studies did not specify their excision approach.

Studies using external radiation, HDR brachytherapy or LDR brachytherapy were compared on study characteristics and study outcomes. See table 2. When analyzing the patient populations per radiation type group, no major differences in patient characteristics were seen.

The mean total radiation dose for studies investigating external radiation and HDR brachytherapy was the same. Studies using LDR brachytherapy applied a higher radiation dose (external: 13.5 ± 3.3, HDR: 13.7 ± 2.6, LDR: 19.3 ± 1.2).

Ref	**< 24 hrs**	**Histol. Confir**	**Def R/K**	**Outcome measures**	**Recurrence**	**L/E**
5mm	yes	no	+/+	- First radiation within 6 hours - pigmentation problems in F5-6 patients	3.1 %	**II**
3-6mm	yes	no	-/-	- 92% had successful treatment	8 %	**III**
5mm	Yes	no	+/+	- Advice 3x6Gy - Better cosmetic results with higher-dose schemes	I: 44 % II: 3 % III: 0 %	**II**
10 mm	yes	no	-/-	HDR brachytherapy can be effective after earlier failure with external radiation	12 %	**III**
10mm	yes	no	+/+	Four fractions within 24hrs 96%: excellent result	3.4 %	**II**
5mm	yes	yes	+/+	- F5-6: high risk of Recurrence - 79% itching ↓ - 87.5% pain ↓	23.6 %	**III**
5mm	yes	no	-/-	-Cosmetic appearance ↑75% -Recurring keloids had received a larger dose	21 %	**III**
2.5mm	yes	no	-/-	Only lineair scars could be treated	19.4 %	**III**

Ref: radiation reference in kiloelectron (keV) and megaelectronvolt (MeV). <24hrs; start of radiation following surgery < 24 hrs. Histologic confirmation of scar characteristics (hypertrophic or keloid scar). Def r/k: Definition of recurrence or keloid scar used in study. L/E: Level of Evidence. According to American Society of Plastic Surgeons Rating Levels of Evidence and Grading recommendations for Diagnostic studies. V: lowest level of evidence, I: highest.

Table 2. Study characteristics analyzed between External radiation, HDR- and LDR brachytherapy

	External radiation	**HDR brachytherapy**	**LDR brachytherapy**
Patients	98.68±102	60±73	140±190
Lesions	106.21±99	66±70	218±304
Location lesion			
- Mixed	53	38	100
- Ear	17	13	
- Non specified	30	49	
Fitzpatrick			
1-6	40	40	33.3
1-2	-	20	-
3-4	8	-	-
5-6	12	-	-
Non specified	40	40	66.7
Radiation (Gy)	13.5±3.3	13.7±2.6	19.3±1.2

HDR brachytherapy was associated with the lowest mean recurrence rate, followed by LDR brachytherapy and external radiation therapy (HDR: 10.5 ± 15; range 0-44, LDR: 21.3 ± 2.1; range 19.4-23.6, external: 22.2 ± 16; range 0-72). When looking only at Level of Evidence type II studies, HDR brachytherapy showed the lowest recurrence rate as well.

Only one study used a device to measure scar quality: Akita et al. described the use of a Durometer to measure scar hardness, which improved with 50% post-treatment compared with pre-treatment.[21] No other studies used objective devices measuring scar elasticity, scar volume or scar colour.

Three studies[5,8,21] used standardized assessment methods as the Patient and Observer Scar Assessment Scale (POSAS)[22,23] or the Vancouver Scar Scale (VSS).[24] Van de Kar et al. reported high POSAS scores (the higher the score, the less the scar resembles normal skin) after treatment using external radiation.[5] In contrast, van Leeuwen et al., reported low POSAS scores after treatment using HDR brachytherapy.[8]

Akita et al reported a significantly better improvement after external radiotherapy on all categories using the VSS compared to pre-treatment.[21] Other studies used different, non-validated, assessment tools.[25]

Short interval

Several authors used a time interval of less than 24 hours between excision and irradiation.[8,26,27] Especially with the use of brachytherapy, authors described an immediate transfer to the radiation department after surgery, resulting in an interval of less than 7 hours.[7,8]

Table 3 shows the differences in recurrence rate for radiation following excision within 7 hours[7,8,13,20,25,28,29], within 24 hours[5,9,11,15,16,30–35] or a longer period between excision and radiation.

In the external radiation group of studies, the rate of recurrence of keloid scars decreased when radiation was applied within 7 hours, compared to 24 hours or longer (external radiotherapy: <7 hrs: 17 ± 4; 7-24 hrs: 28 ± 7; >24 hrs; 21 ± 2). With HDR brachytherapy, radiation within 7 hours showed no difference in recurrence rate compared with HDR brachytherapy applied within 24 hours. Within the LDR brachytherapy group comparison was not possible because of the low number of included studies.

Table 3. Recurrence rate of the different radiation types related to the time interval between surgery and adjuvant radiotherapy

	Hours		
	>24	**7-24**	**< 7**
External	21.5 ± 2.3	28.4 ± 7.3	16.8 ± 4.3
Brachy HDR	-	10 ± 2	10.7 ± 8.3
Brachy LDR	-	19.4*	22.3 ± 1.3
Total	21.5 ± 2.3	25 ± 6	14 ± 4

Time interval between surgery and radiotherapy of more than 24 hours, less than 24 hours and within 7 hours.*; only one study.

Single-fraction

Of the included studies, Ragoowansi et al.[11,16] and Sclafani et al.[13] promoted a single-fraction radiation therapy using external radiation. When looking at the mean recurrence rate for these single-fraction protocols, a lower recurrence rate (12 ± 8.8) was seen compared to the mean recurrence rate within the total external radiation group (22.2 ± 16). In addition, no complications were described and good results were achieved in terms of scar quality and patient's satisfaction.

Recurrence

Ten studies (30%) provided information about the incidence of recurrence. The mean time for the incidence of recurrence after treatment was 14.8±6.7 months with a range of 2 to 36 months. See table 4. Twelve studies described a definition for recurrence. Authors defined recurrence as; any regrowth of tissue[25,81,13,36,37]; mild or failure relapse[38]; a symptomatic reappearance[28]; a regrowth extending beyond the original surgical field[5]; pain, itch from the scar, clinical evidence of a mass; obvious return of the lesion[11,16] or just as; impairment.[35]

Tabel 4. Recurrence percentages in months

Study	Recurrences		
Arnault et al., 2009	84%	->	24
	16%	->	36
	Mean	->	26
Darzi et al., 1992	67%	->	6
	22%	->	12
	11%	->	24
	Mean	->	9
Doornbos et al., 1990	70%	->	6
	19%	->	12
	9%	->	24
	Mean	->	9
Escarmant et al., 1993	90.8%	->	12
Ollstein et al., 1981	75%	->	12
	12.5%	->	24
	12.5%	->	36
	Mean	->	16
Perez et al., 2001	Mean	->	12
Sclafani et al., 1996	Mean	->	17
Wagner et al., 2000	15%	->	2
	45%	->	6
	16%	->	12
	24%	->	24
	Mean	->	11
Kovalic et al., 1989	Mean:	->	18
Ragoowansi et al., 2003	Range:	->	12-60

Complications

In all selected studies, no relation between scar radiation and malignancies was found. This is in accordance with other literature.[39–41]

Discussion

The use of excision followed by adjuvant irradiation for the treatment of keloid scars is mostly based on research performed in the 1960's by Van den Brenk and Cosman[26,27] They were the first to compare different radiation protocols for the treatment of keloid scars.[26,27]

In their studies, the treatment options were divided in two categories: 1) primary irradiation without surgery and 2) lesions treated by excision combined with planned early and late prophylactic irradiation. Both authors draw comparative conclusions stating that: A) Primary irradiation without surgery may relieve symptoms, but fails to cause resolution of the actual lesion. B) Late postoperative radiation is associated with higher recurrence rates compared to early postoperative radiation.[26,27]

In 1967, Nicoletis et al. was the first to introduce interstitial (or internal) radiation, also called brachytherapy.[42] Hereby, radiation is effectively localized inside the scar lesion, only targeting the area which is desired to irradiate. This in contrast to external radiotherapy in which considerable radiation of adjacent tissue is inevitable. This is undesirable, since exposure to radiation should be minimized in this often young population suffering from a benign disease which only needs radiation in a small area.

The conclusions of van den Brenk and Cosman[26,27] combined with the introduction of brachytherapy, led to several recent publications in which protocols were described resulting in low recurrence rates and enhanced patient convenience.[9,14,24,29,43] These protocols used HDR brachytherapy in one or more fractions, applied immediately after excision. We evaluated these results and recommendations with a comprehensive review based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).

Radiation modality

External radiation resulted in a higher recurrence percentage compared to HDR and LDR brachytherapy. This can be explained by the fact that brachytherapy, in contrast to external radiation: 1) Has more focused and efficient radiation of the targeted area 2) Requires a lower dose of radiation to achieve the same therapeutic effect, thereby reducing radionecrosis; 3) Provides less irradiation to surrounding healthy tissue.

When looking at brachytherapy, HDR brachytherapy scored lower recurrence rates compared to LDR brachytherapy. Although both techniques are considered as brachytherapy, with HDR brachytherapy the total radiation is given in several minutes and ends shortly

(<24 hours) following the operation. With LDR brachytherapy however, this dose is spread out over 20 to 72 hours, which is actually a delayed treatment. In addition, HDR brachytherapy is an out-patient procedure enhancing patient convenience, whereas LDR brachytherapy requires hospitalization in lead coated radioprotection chambers.

Short interval

Van den Brenk and Cosman[26,27] showed that early irradiation, within 24 hours, results in lower recurrence rates, compared to a more delayed irradiation. Other authors, however, hypothesized that early radiation within 7 hours could lower the recurrence rate even further.[8] As table 3 shows, this hypothesis was confirmed with the external radiation group of studies.

Surprisingly, this hypothesis was not confirmed in the HDR brachytherapy group. Notably, this discrepancy was caused by one study, which showed a very high recurrence percentage.[29] When analyzing this RCT study of Veen and Kal, there were 3 groups receiving different radiation doses within 6 hours.[29] The group receiving the smallest amount of gray (10Gy), scored a high recurrence percentage of 44%. The other two groups in this study receiving 14Gy and 18Gy showed a 3% and 0% recurrence rate, respectively. The authors hypothesized that this high recurrence rate was due to the low radiation dose of 10Gy. This is in accordance with other HDR brachytherapy studies which all applied a dose of 12Gy or more. Thus, when excluding this deviant rate, an average recurrence rate of 2.3% was seen in with studies applying brachytherapy within 7 hours. This confirms the trend already seen in the external radiation group towards a low recurrence rate when irradiation is applied within 7 hours. Also, it may show that a minimum of 12 Gy irradiation is required.

The mechanism behind immediate irradiation following scar excision remains unclear. Many studies explain the effect of irradiation by the prevention of keloidal fibroblasts to repopulate.[44,45] This seems illogical, since extralesional scar excision already removed all keloid fibroblasts. Another explanation could be that surgical scar excision will attract local fibroblasts. Stimulated by humoral or cellular factors, these local fibroblasts lead to a disturbed proliferation homeostasis, which eventually can lead to recurring of the scar. Irradiation may modulate these humoral or cellular factors, leading to a disruption of this cascade, thereby preventing scar recurrence. Since this process starts directly after the operation, it is important to start the irradiation as quickly as possible, i.e. transferring the patient immediately after surgery to the radiation department.

Single-fraction

Surgical excision followed by a single-fraction radiation dose would prevent repeated (outpatient) consults, thereby increasing the patient convenience and therapy adherence. More-over, van den Brenk et al. stated that there is no place for the use of fractionated small doses of radiation to attain a larger cumulative dose.[26] They state that the dose-effect relationship is strictly threshold and that doses of less than 10Gy substantially fail to inhibit the growth of regeneration of the scar.[26] Out of the studies in this review, 3 studies administered a single-fraction dose with external radiation. They showed low recurrence percentages with good results in terms of scar quality and patient's satisfaction. Importantly, no complications were noted.[11,13] In our opinion these results are promising. However, a RCT is required to confirm these results and prove the safety and efficacy of a single-fraction radiation therapy.

Study protocol

A large part of the initially selected studies were excluded because they did not describe the minimum follow-up of the study. This review reports a mean scar recurrence after a mean of 15 months post-treatment with a maximum range of 36 months. Therefore, we recommend a minimum of 15 months follow-up, but preferably a period of 2 or even 3 years.

In addition, most studies did not clearly define keloid characteristics, keloid recurrence and study outcomes. Defining keloid characteristics prevents inclusion of non-keloid scars such as hypertrophic scars. This is relevant since hypertrophic scars have better prognostic factors than keloid scars. We advise to use the following definition for inclusion of keloid scars as stated by Ogawa[18]: "A fibroproliferative disorder of the skin that grows beyond the boundaries of the original wound or has an unrecognized origin". Also, post-treatment histology of the excised lesion may be used to confirm the nature of the scar.

As described in the result sections, only 12 studies (36%) defined keloid scar recurrence. Most studies, evaluating scar recurrences use the definition as stated by Cosman and Wolff in 1974[19]: "A growing, pruritic, nodular scar constituted a recurrence; a flat, nonpruritic scar was considered a good result". Furthermore, to define the (residual) scar quality, validated measurement devices can be used. Examples are the Cutometer for scar elasticity[46], the Dermaspectrometer for scar colour[47] and the Patient and Observer Scar Assessment Scale for general scar assessment.[22,23] Finally, inclusion of a variety of Fitzpatrick (F1-6)[48] score patients is preferable since Afro-American patients (F5-6) are more prone to pigmentation disorders[49,50] and scar recurrence[28] compared to Caucasian patients (F1-3).

Primary closure

The recommendations in this manuscript are based on the included studies, which included all keloid subtypes that could be closed primarily after excision. In the case of large or high-tension keloid scars, which cannot be closed primarily, we advise to use skin grafts as described by Li et al.[51]

Comparison with other treatment modalities

As demonstrated in this systematic review, excision with adjuvant HDR brachytherapy offers total scar eradication with low recurrence rates (mean; 10.5%). Other treatment modalities will not always result in a complete volume reduction and demonstrated higher recurrence rates; corticosteroid injections monotherapy (>50%[18]), surgery with corticosteroid injections (<50%[3]) and Intralesional cryotherapy (24%[52]). On the other hand, surgical excision with irradiation is not always possible due to the patient characteristics (pregnant or age; <12 yr) or the location of the keloid scar (radiosensitive locations such as the thyroid gland). Finally, it should be mentioned that the costs of excision with irradiation exceed the costs of other treatments significantly. Therefore, excision with adjuvant radiation therapy should be regarded as a 'last resort' for (recalcitrant) large keloid scars, when other non-surgical treatments have failed.

Conclusion

Based on this systematic review of the literature, the use of HDR brachytherapy, preferably applied within 7 hours, results in a low recurrence rate. Also, single-fraction irradiation appears safe and enhances patient convenience. However, the quality of this evidence is limited; There is a paucity of high quality studies with clearly defined methods and study outcomes. More randomized studies comparing different radiation protocols, will generate stronger recommendations.

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Chapter 9

High-dose-rate brachytherapy for the treatment of recalcitrant keloids: a unique, effective treatment protocol

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Abstract

Background

Keloids cause aesthetic disfigurement and physical complaints, mainly pain and pruritus. Treatment of these scars is difficult, with high recurrence rates forming the main issue. Surgical excision with adjuvant radiotherapy is considered the most efficacious treatment. At our institution, we have been treating keloids with a high-dose-rate (HDR) brachytherapy procedure for over 10 years, using a protocol with the lowest total radiation dosage known in the literature.

Methods

This prospective study included 43 patients of all Fitzpatrick skin types, with 67 keloids in total. After extralesional excision, a radiation scheme of 2x6Gy was administered in 2 fractions: the first within 4 hours after surgery, the second within 24 hours. Scars were measured and recurrence was judged. Scar appearance was evaluated using the Patient and Observer Scar Assessment Scale.

Results

The recurrence rate was 3.1% at a mean follow-up of 33.6 months. A significant average scar surface decrease of 56.7% was measured, p=0.01. Complaints of pain and pruritis decreased with 82.9% and 87.2% respectively. Patients were satisfied with the treatment in 88.6% of the cases and in 77.1% with the cosmetic result. Pigmentation problems were seen in 21.4% of the scars, mostly in Fitzpatrick type 5-6/Afro-American individuals.

Conclusions

The results of this prospective study show a good cosmetic outcome with a low recurrence rate. The unique radiation schedule proves the efficacy and safety of HDR brachytherapy and suggests the importance of immediate postoperative radiation. In addition, only one out-patient treatment is required after surgery, enhancing patient convenience.

Introduction

Keloids are benign fibrous cutaneous tumours, that arise as a result of abnormal healing of the dermis.[1] Besides aesthetic disfigurement, keloids can cause major physical complaints of pain and pruritus, thus impairing the quality of life of the patient.[2–5] Men and women are equally affected in incidence, but the incidence is 5 to 15 times higher in dark-skinned individuals.[1,6–8] Treatment of keloids is a significant challenge for the clinician, because no single treatment modality has proven widely effective in preventing recurrence.[1] According to the international advisory panel on scar management, surgical excision with post-operative radiation therapy is considered the most efficacious treatment.[4] Radiation is applied through traditional external beam radiation therapy (EBRT), low-dose-rate (LDR) brachytherapy or high-dose-rate (HDR) brachytherapy. Brachytherapy, also known as internal irradiation, is a form of radiotherapy in which a radiation source is placed inside the target area. It offers several practical advantages over EBRT, including improved dose optimization, better radioprotection to surrounding healthy tissue and a more focused radiation distribution and delivery.[9] In addition, brachytherapy shows lower recurrence rates (3.4% to 23.6%) compared to external beam radiation (12% to 27%).[10–12] LDR brachytherapy, in which a radioactive seed is implanted in the target area for a certain period, requires patients to be hospitalized for several days, due to the relatively long treatment time. In contrast, with HDR brachytherapy, a radioactive source is directed through a hollow catheter into the target site reducing treatment time to typically only several minutes. This process can be repeated several times in an out-patient clinical setting. Therefore, there is no need for hospitalization in lead coated radioprotection chambers, as is required with LDR brachytherapy. At our institution, we have been treating keloids with a HDR brachytherapy out-patient procedure for over 10 years. The treatment protocol employs a unique radiation schedule with a total dose of 12 Gy. This is the lowest dosage known in literature for the treatment of keloids. This long-term prospective study evaluated the effectiveness of this radiation schedule in a large population including patients of all Fitzpatrick skin types. Scars were evaluated with respect to local recurrence and cosmesis. In addition, scar evaluation was performed with the Patient and Observer Scar Assessment Scale.[13-15]

Patients and methods

All patients presented with keloids in the period 2003-2009 were included in a prospective study according to the following inclusion criteria: Firstly, a keloid was defined as excessive scar tissue raised above skin level and proliferating beyond the confines of the original lesion.[1,16,17] Keloids were distinguished from hypertrophic scars based on the clinical judgment of experienced plastic surgeons and on the age of the scar (>1yr).[18] Secondly, the keloid had proven insensitive to at least one other treatment modality. Lastly, patients had to be older than 10 years of age. Exclusion criteria contained: keloids not suitable for excision with primary closure due to its size or anatomical location, pregnancy or diabetes. All patients were treated at the Plastic Surgery and Radiation Oncology departments of the VU University medical center, Amsterdam, the Netherlands. Before treatment, all patients were consulted by both surgeon and radiotherapist, gave informed consent and agreed to radiation therapy treatment and follow-up. The study was approved by the medical ethical council of the VU University in the Netherlands.

Treatment protocol

The keloid was marked and excised extralesionally under local anaesthesia (figure 1). Hemostasis was achieved with electrocautery. Subsequently, a metal tipped Varisource catheter (external diameter: 1.6 mm, length: 150 cm, Varian medical systems, Palo Alto, USA) was positioned between the dermal edges of the entire wound, 5 mm below the surface of the skin and extending out of the skin beyond the wound at both sides. The catheter followed the shape of the scar, and was kept intact without kinking. The wound was closed primarily using Monocryl 4-0 or 5-0 intracutaneously and subcutaneously, thereby also fixating the catheter against the dermis. Postoperatively, patients were transferred immediately to the radiation department, where the catheter was connected to the Iridium-192 remote control afterloader (Varisource, Varian medical systems, Palo Alto, USA). The planning target volume was defined as a cylinder along the axis of the scar, with a central diameter of 5 mm. Patients received 2 fractions of HDR brachytherapy. Within 4 hours after the resection, the first brachytherapy fraction of 6 Gy at 5 mm of the source axis was given. This dose was prescribed at the middle of the catheter and continuous dwell positions were used from the skin entry point to the skin exit point of the catheter. Equal dwell times were used for all dwell positions. Within 24 hours after the first fraction, a second fraction of 6Gy was administered to a total dose of 12Gy in 2 days. The typical radiation time was 100s, depending on the decay of the source. After the second fraction the catheter was gently removed. Patients received no other adjunct treatment for their keloids, such as silicone gel sheeting, scar cream, pressure clothing or steroids.

Follow-up

Before surgery, age, gender, date of treatment, original scar size, scar etiology, scar duration, previous treatments, skin type in Fitzpatrick score,[19] anatomical localization, clinical aspects, and brachytherapy parameters were scored and photos were taken. Patients were seen at postoperative consultations for long-term follow-up at the VU medical center. During consultation, the keloid location was identified using a well-documented photographic archive and precise wound descriptions. The residual scar was examined, photographs were taken and surface area was measured. The recurrence rate was used as primary outcome and was defined as a growing, pruritic, nodular scar as described by Cosman and Wolff.[20] Subjective scar evaluation was performed at follow-up by the patient and an independent medical doctor using the Patient and Observer Scar Assessment Scale as a descriptive measure to record the postoperative scar.[13-15] Each item of this scale was scored using a 10-step score, in which 10 reflected 'worst scar imaginable' and 1 indicated 'normal skin', amounting to a total score of 60.[13-15] To calculate the overall score, all 6 items were added amounting to a total score of 60. In addition, the patients were questioned on 7 predetermined parameters: pain, pruritus, movement induction, burning sensation, physical burden, functional discomfort and dysesthesia.

Figure 1. Surgical procedure. The keloid (left) was excised extralesionally (right) and a catheter was positioned between the dermal edges of the wound, 5 mm below the surface of the skin and extending out of the skin beyond the wound (below). The catheter followed the shape of the scar, and was kept intact without kinking. Postoperatively, patients were immediately transferred to the radiation department

Statistical analysis

All statistical analyses were conducted using SPSS version 20.0 (SPSS, Inc., Chicago, Ill.) Dependent t-tests were performed to compare the VAS scores before and after treatment.

Descriptive statistical analyses were used to assess outcomes. A p-value < 0,05 was considered statistically significant.

Results

Characteristics

Between 2003 and 2009, 43 patients with 67 keloids were treated. All patients started the study, but during follow-up some patients were untraceable (n=8) or no longer willing to participate in the study (n=7). At long term follow-up, a total of 28 patients with 35 keloids were analysed, constituting 65% of the initial 43 patients (figure 2).

Figure 2. Patient flowchart. Between 2003 and 2009, 43 patients with 67 keloids were treated. In total, 28 of the initial 43 patients with 35 keloids participated in this study.

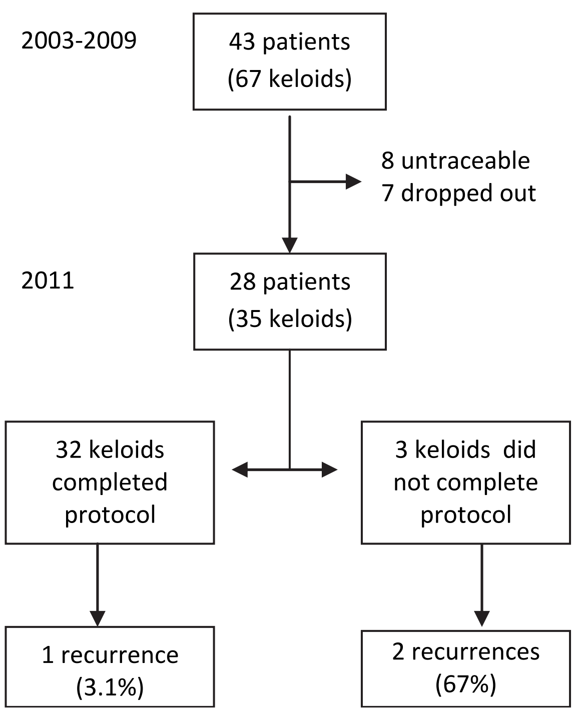


Table 1 demonstrates the patient characteristics of the study group. The mean age of the patients was 36.3 years (range 18-68), 66% were female and 34% male patients. 34.3% of the patients were Caucasian (Fitzpatrick 1-2), 14.3% had Mediterranean/Asian skin colour (Fitzpatrick 3-4) and 51.3% were Afro-American (Fitzpatrick 5-6). The mean age of the scars was 6 years (range 2-21) and all scars had proven recalcitrant to other treatments, but none received prior radiation therapy. Thirty-seven percent of the keloids were located on the ear lobules and 20% on the sternum.

Table 1. Patient characteristic and results

					Patient characteristics				Results
Scar	**Age (yr)**	**Sex**	**Duration (yr)**	**Location**	**Pre-treatment**	**Cause**	**Fitzpatrick score**	**Recurrence**	**Side effect**
1	20-40	F	2	Shoulders	EXC, ST, SIL	Acne	5-6	No	Hypopigmentation
2	20-40	M	5	Retroauriculair	EXC, SIL	Surgery	3-4	No	None
3	20-40	F	5	Retroauriculair	EXC, SIL	Surgery	1-2	No	None
4	20-40	M	3	Ear lobules	EXC, ST	Trauma	1-2	No	None
5	20-40	F	4	Ear lobules	ST	Ear piercing	1-2	No	None
6	20-40	F	10	Ear lobules	EXC, ST	Ear piercing	1-2	No	None
7	≥60	M	5	Limb	EXC, ST, SIL	Unknown	5-6	No	None
8	40-60	F	8	Upper back	ST, SIL	Trauma	5-6	No	None
9	40-60	M	8	Upper back	ST, SIL	Trauma	5-6	No	None
10	20-40	F	5	Ear lobules	SIL	Ear piercing	1-2	No	None
11	20-40	F	6	Sternum	ST, SIL	Insect bite	1-2	No	None
12	20-40	M	18	Sternum	EXC, ST, SIL	Unknown	1-2	No	None
13	20-40	F	19	Sternum	ST	Acne	5-6	No	Hyperpigmentation
14	40-60	M	5	Head	EXC, ST, SIL, CRYO	Unknown	5-6	No	None
15	20-40	F	5	Head	EXC, ST, SIL, CRYO	Unknown	3-4	No	Hypopigmentation
16	20-40	M	5	Head	EXC, ST, SIL, CRYO	Unknown	3-4	No	None
17	20-40	F	4	Ear lobules	EXC, ST, SIL	Ear piercing	5-6	No	None
18	20-40	F	3	Other	ST	Trauma	5-6	Yes	None
19	20-40	F	3	Other	ST	Trauma	1-2	No	None
20	20-40	F	12	Shoulders	ST, SIL	Acne	3-4	No	None
21	40-60	F	1	Ear lobules	EXC, ST	Unknown	1-2	No	None
22	40-60	F	1	Sternum	ST, SIL	Surgery	5-6	No	Infection
23	≥60	F	2	Ear lobules	EXC, ST, SIL	Trauma	5-6	No	None
24	40-60	F	20	Sternum	EXC, ST, SIL, CRYO	Acne	5-6	No	None
25	20-40	F	2	Shoulders	EXC, ST	Surgery	5-6	No	None
26	20-40	F	2	Ear lobules	ST	Surgery	5-6	No	None
27	20-40	M	2	Ear lobules	ST	Surgery	3-4	No	None
28	20-40	F	14	Sternum	ST, SIL	Surgery	1-2	No	Infection
29	20-40	M	3	Ear lobules	EXC, ST, SIL	Ear piercing	5-6	Yes	None
30	40-60	M	3	Sternum	EXC, ST, SIL	Surgery	1-2	No	Telangiectasis
31	≥60	F	4	Thorax	EXC, ST, SIL	Acne	5-6	No	Hyperpigmentation
32	20-40	F	2	Ear lobules	EXC	Surgery	5-6	No	Hyperpigmentation
33	0-20	M	1	Ear lobules	EXC, ST	Ear piercing	5-6	No	Hypopigmentation
34	40-60	F	2	Abdomen	None	Surgery	5-6	No	None
35	20-40	M	16	Ear lobules	EXC, ST	Ear piercing	1-2	Yes	None

F: female, M: male, Cryo: cryosurgery, EXC: surgical excision, ST: intralesional steroids, SIL: silicon gel/sheeting.

Radiation protocol

Three patients did not complete the radiation protocol (figure 2). Two of these three patients had an overnight dislocation of the catheter; one of which received an EBRT session of 6,6Gy at day 2, instead of the 6Gy HDR brachytherapy. The other did not receive any treatment at day 2. The third patient had a lower dose HDR brachytherapy during his second session, due to a technical problem. These 3 patients were not excluded from the study.

Recurrence

Out of the patients that completed the radiation protocol, one recurring scar (3.1%) was seen at a mean follow-up of 33.6 months (range: 24 to 96 months). Of the 3 patients that did not complete the radiation protocol, 2 showed recurring scars (66.6%) (figure 2).

Surface area

The average keloid surface area before treatment was 11.44 cm^2 (width: 2.2 ±.2 cm, length: 5.2 ±.8 cm). After treatment, at long-term follow up, the average surface area of the non-recurring keloids had been reduced to 4.95cm^2 (width: 0.9 ±.2 cm, length: 5.5 ± 1 cm), illustrating an average decrease of 56.7%, p = 0.011 (figure 3). The scar tissue that remained, was the result of skin widening due to postoperative radiation scar weakness and the high tension at the location of the excised keloids.

Patient and Observer Scar Assessment Scale

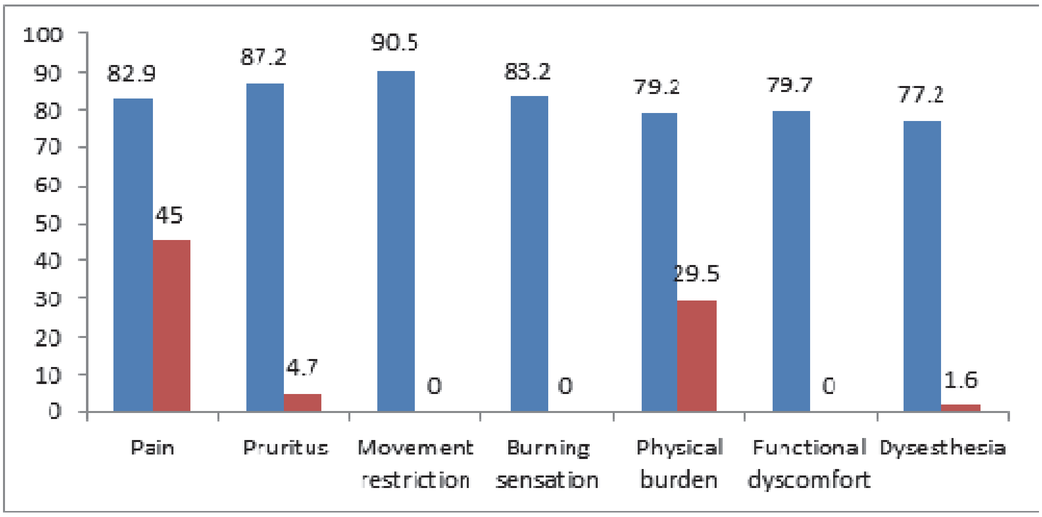
The scar evaluation using the Patient and Observer Scar Assessment Scale showed low scores according to both patients and doctor on the keloids after treatment at long term follow-up (the lower the score, the more the scar resembled normal skin). The doctor scored an overall value of 16.71 ± 1.1, and the patient a slightly higher score of 19.69 ± 2.4 (0; normal scar, 60; worst scar). With respect to physical complaints, all 7 parameters improved in the majority of the non-recurring patients. On average, pain decreased with 82.9%, pruritus with 87.2%, movement restriction with 90.5%, burning sensation with 83.2%, physical burden with 79.2%, functional discomfort with 79.7% and dysesthesia with 77.2% (figure 4). Recurring scars showed less or no improvement. Eighty-six percent of all patients were satisfied with the treatment and 77% were satisfied with the cosmetic result. 88.6% would repeat the treatment if necessary.

Complications and adverse events

Complications included postoperative infection (5.7%) requiring oral antibiotics. Problems with hypo- and hyperpigmentation were seen in 6 out of 35 scars (21.4%) during follow-up.

When subdividing this group into skin colour, 5 of these patients had a Fitzpatrick type 5-6 skin (Afro-American) and 1 patient had a Fitzpatrick type 3-4 skin (Mediterranean/Asian). No cases of radiation-induced wound dehiscence, dermatitis, neuritis or cutaneous malignancy appeared.

Figure 3. A questionnaire was used to determine patient improvement in seven physical complaints in a percentage fashion compared before and after treatment, subdivided into patients without scar recurrence (blue) and patients with scar recurrence (red)



Discussion

This prospective study shows that keloid excision followed by a 2x6 Gy HDR brachytherapy schedule resulted in significant scar surface reduction, alleviated complaints of pain and itching, provided good cosmetic results and yielded a low recurrence rate of 3.1%. Finding a successful treatment for recalcitrant keloids is of great importance as keloids cause aesthetic disfigurement and physical complaints, mainly pain and pruritus. Thus far, treatment of recalcitrant keloids has proven challenging with high recurrence rates and even growth stimulus as the main issue.[1] Excision with adjuvant radiotherapy is considered the most efficacious treatments for keloids.

Comparison with existing literature

As stated by Arneja et al.[9] brachytherapy offers several practical advantages over EBRT, including improved dose optimization, better radioprotection to surrounding healthy tissue and more focused radiation distribution and delivery. Comparison between EBRT and brachytherapy was difficult, because most studies were retrospective trials, often not distinguishing clearly between hypertrophic and keloid scars, lacking a variety of Fitzpatrick skin type patients and not always respecting the minimum follow-up term of one year.[21] In this prospective trial we used a frequently cited recurrence definition as set by Cosman and Wolff.[20] In addition, we evaluated scars pre- and postoperatively using the Patient and Observer Scar Assessment Scale, which is validated for assessing keloid scars.[13-15]

Recurrence

After a mean follow-up of 33.6 months, we found a low recurrence rate of 3.1%. Previous studies have stated recurrence rates ranging from 12-27% following EBRT[11,22,23] HDR brachytherapy, as in this study, has been reported to yield lower recurrence rates. Veen and Kal found a 3% recurrence rate in a 1 x 6Gy + 2 x 4Gy schedule and a 44% recurrence rate in a 1 x 4Gy + 2 x 3Gy schedule,[24] but the latter schedule included only 9 patients.[24] Arneja et al. used a 3 x 5Gy schedule in 25 patients and found 8% recurrence,[9] whereas Guix et al. used a 6 x 3Gy schedule and found 4.7% recurrence in a large population of 169 patients.[25] However, the radiation dose of Guix et al. was prescribed at 1.0 cm distance from the source axis, which corresponds to a total dose of about 6 x 6 Gy at 0.5 cm of the source axis.[25] Kuribayashi et al. used superficial brachytherapy with a 4 x 5Gy schedule and found 9.7% recurrence in 21 patients.[26] Finally, Garg et al.[27] found a 12% recurrence with a 3 x 5Gy schedule in 12 patients, but all patients had previously received EBRT.[12]

Radiation protocol

The radiation protocol as used in this study (2 x 6Gy administered in 2 fractions; the first within 4 hours post surgery and the second within 24 hours) has never previously been published.[4,9] We believe that the radiation dose and schedule have contributed to the low rates of recurrence reported. Firstly, a low total radiation dose limits damage to healthy surrounding tissues and reduces the risk of complications, two factors that could otherwise contribute to a higher recurrence rate. Secondly, we find the timing of the first radiation to be of great importance. While other studies mostly began the first procedure within 24 hours[12,25,26] our first fraction was given within 4 hours. We believe that immediate postoperative adjuvant radiotherapy radiation prevents immune cells from invading and proliferating into the lesion reducing recurrence. This hypothesis is supported by several other groups treating keloids immediately after surgery or even perioperatively.[27-29]

Interestingly, radiation to prevent heterotopic ossification is nowadays even administered pre-operatively instead of postoperatively after extensive research by Kantorowitz.[30–32] Finally, this radiation schedule required patients to return to the outpatient clinic only once for the second radiation treatment, enhancing patient convenience.

Cutaneous malignancy

Since we used a relatively low radiation dosage, complications such as radiation-induced wound dehiscence, dermatitis, neuritis or cutaneous malignancy were not seen. Although one might assume a small theoretical risk of developing radiation induced malignancy to exist, to our knowledge no treatment related cancers have been reported in keloid management literature.[33-35] In addition, because the theoretical risk of tumor induction is dependent on the total irradiated volume, we used brachytherapy instead of EBRT in which the volume is typically much larger.

Pigmentation changes and physical complaints

Pigmentation changes (both hypo- and hyperpigmentation) were reported in 21.4% of the patients at long term follow-up. Other studies reported equal or lower rates of pigmentation problems (0-17%).[9,12,24-26] Importantly, publications describing low rates of pigmentation problems included mostly patients of Fitzpatrick 1-4 skin type.[12,24,25] Our data shows that the incidence of pigmentation problems is higher in Afro-American patients (Fitzpatrick 5-6) than in Fitzpatrick 1-4 skin type patients.[22,36] Although physical complaints improved in the majority of the patients, a percentage of the patients still experienced (some) physical complaints of pain and itching at long-term follow-up. This is compatible with most other studies and it is unclear whether this is the result of residual scar tissue or recurrence, since these complaints are also known as side effects from skin irradiation.[18,29,34,37]

Two- or three-dimensional treatment plans

Other groups described the use of two- or three-dimensional treatment plans in order to locate the source and to achieve dose optimization.[9] In our opinion, pre-treatment radiographs or even CT scanning would make the procedure very time consuming, and cost expensive. In addition, it was found that in experienced hands, catheter placement and fixation would ensure well coverage of the target volume by radiation.

Limitations

Although the loss of follow-up is comparable to other studies, we acknowledge that failure of follow up could imply a bias, since patients with good results may be less motivated to return for follow-up.[3,18,34,37] Another limitation of this study is the lacking of randomization. On this account, our group is currently performing a multicenter randomized study including brachytherapy, intralesional cryotherapy and intralesional corticosteroids.[38]

Conclusion

In conclusion, the results of this study show HDR brachytherapy with a 2 x 6Gy dosage to yield good cosmetic outcomes with low recurrence rates. The unique schedule confirms the efficacy and safety of HDR brachytherapy and suggests the importance of immediate postoperative radiation. In addition, only one out-patient treatment is required after surgery, enhancing patient convenience.[39-41]

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Chapter 10

Reply: High-Dose-Rate Brachytherapy for the Treatment of Recalcitrant Keloids: A Unique Effective Treatment Protocol

Michiel van Leeuwen
Suzanne Stokmans
Otto Meijer
Anne-Eva Bulstra
Paul van Leeuwen
Frank Niessen

Plastic and Reconstructive Surgery 2015;135(4):792-793e.

Dear Sir,

We read with great interest the article of van Leeuwen MCE entitled “High-Dose-Rate Brachytherapy for the Treatment of Recalcitrant Keloids: A Unique, Effective Treatment Protocol”.[1] The authors presented a novel radiation regimen, which added another arsenal in a plastic surgeon’s armamentarium in managing recalcitrant keloids.

Close examination of the study raised some questions that might compromise accurate interpretation of results, which we would like to communicate with the authors.

As the authors described the inclusion criteria that the keloid had proven insensitive to at least one other treatment modality, NO. 34 in Table 1 with none pretreatment should be excluded, which could be a confounding problem in this study.

For the “Complications and Adverse Events” part, problems with hypopigmentation and hyperpigmentation were seen in “six of 35 patients “should be corrected into “six of 28 patients”, which may confuse the readers. And we believe it is purely a typographical error.

In addition, keloids not suitable for excision with primary closure are excluded from this study because of the technical limitation. Here we want to introduce a novel method we had applied to patients with large keloids called “Keloid Edge Precut, Preradiotherapy Method”.

Briefly, an incision was made down to the subcutaneous layer around the edge of the keloid, and radiotherapy (9Gy) was applied on the following day. Then the keloid was removed, and the wound was closed using a skin graft. Radiotherapy (9Gy) was applied for the second time when the graft was found to have survived. Lower recurrence rate and higher aesthetic satisfaction could be achieved when compared to the conventional postoperatively radiation protocol.[2]

After the total excision of keloids, radiation therapy has been demonstrated as one of the most effective treatment methods to prevent recurrence.[3] High-dose-rate brachytherapy produce better results for keloids reported in this study is consistent with other studies.[4,5] But the radiation protocol used in this study is completely new. Although a variety of Fitzpatrick skin type patients were included in this study, only 25 patients had completely followed the radiation protocol may probably lead to a bias. Randomized controlled studies are urgently needed to achieve solid clinical data.

Feil Long
Xiojun Wang

Plastic and Reconstructive Surgery 2015;135(4):791-2e.

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Sir,

We have read with interest the letter of Drs. Long and Wang. First, we would like to confirm the addressed comments of the authors. Patient 34 did not receive previous treatments and should have been excluded. Problems with hypopigmentation and hyperpigmentation were seen in six of 35 scars, instead of 35 patients.

Second, the protocol described in our article is only applicable if the wound can be closed primarily following excision of the lesion. This is required to enclose and fixate the brachytherapy catheter (see figure 1 of the article[1]). It is therefore impossible to apply brachytherapy to large keloids that cannot be closed primarily. These scars, however, could be closed with a skin graft, after which external irradiation can be applied as described in the protocol of Dr. Li et al.[2]

Finally, Drs. Long and Wang discussed the possibility of bias, because three patients did not complete the treatment protocol. However, the three patients were excluded from the results. Therefore, no bias was possible. Thus, when looking into figure 2 of the article, 32 keloid scars completed the treatment protocol, with a recurrence rate of 3.1%. We agree with Drs. Long and Wang that larger randomized studies are more than welcome.

Michiel van Leeuwen
Suzanne Stokmans
Otto Meijer
Anne-Eva Bulstra
Paul van Leeuwen
Frank Niessen

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Chapter 11

General discussion

Discussion and future perspectives

This chapter discusses the main findings in this thesis and concludes with recommendations for future research and clinical practice. The aim of this thesis was to evaluate current scar treatments. It studied the use of dermal substitutes for the treatment of acute burns and reconstructive wounds (Part 1). Also, it evaluated current treatment modalities for keloid scars (Part. 2).

Part 1. Discussion of burns

In the last decades, improved medical health care decreased the mortality in patients with extensive deep burns.[1] As a result, more patients survived. However, large burn traumata still result in severe functional and aesthetic skin defects. Therefore, the improved healthcare raises new challenges in modern wound care.[1]

Momently, autografting is still the gold standard for the treatment of full thickness burn wounds. With this treatment, harvesting the split thickness skin graft (SSG) causes new lesions on the patient. Additionally, the burn wound itself often heals with severe scarring after grafting with SSG's. The lack of dermal tissue, as with SSG's, is now thought to be responsible for the reduced quality of the healed skin or scar following autografting.[1,2] Residual dermis or dermal elements may minimize contraction and scar formation.

When looking into the current available dermal substitutes, it is still the case that each of the three main classes of dermal substitutes had specific properties that made it both suitable and problematic for dermal substitution. Evaluating the dermal substitute named Matriderm showed improved scar elasticity after 4 months and enhanced scar appearance after 1 year.[3,4] Also, there was still an elasticity increase after dermal substituted scar compared to non-substituted scars twelve years post-treatment.[5] Subjective assessment showed several outcomes in favor of the substituted scars.

Remarkably, the substituted scars showed a smoother aspect of the skin compared to the non-substituted scars. This was confirmed by an improvement in favor of the substituted scars (reconstructive wounds) as measured with the PRIMOS device. This improvement may be explained by the formation of hypertrophy in the interstices (gaps) of the grafts: With non-substituted scars it is seen that the interstices of the grafts heal with hypertrophy formation, which leads to a disturbed texture of the scar (see figure 2B of the General Introduction). A dermal substitute possibly bridges the interstices of the graft resulting in a smoother aspect of the skin.

In conclusion, this thesis displayed the successful implementation of a dermal substitute: Matriderm was designed according to the general principles for adequate functioning of dermal substitutes and showed effective optimization of scar quality. This is important, since (long-lasting) improvement of scar quality may reduce the need for reconstructive surgery following burn trauma, possibly justifying its additional (high) costs.

Future directions of burns

Although dermal substitutes optimized scar quality, it does not lead to scar free healing. To reach the ultimate goal of skin repair without scarring, further development in skin substitutes is required. Ideally, skin substitutes contain both a dermis and an epidermis. Also, other structures should be added: sweat glands, hair follicles, sebaceous glands and nerves.

Currently, stem cell therapy is considered the best potential option for generating the above named structures and elements of the skin.[1,6–8] However, clinical implementation of stem cells has been disappointing so far, due to poor persistence and survival rates of the transplanted stem cells.[1,6–8] Future directions should therefore be based on the development of three-dimensional artificial skin substitutes. The three-dimensional skin substitutes will be able to act as an ideal scaffold environment for hosting stem cells and enabling the influx of cells that will function as dermal cells.[6,8]

Another development concerns the treatment strategy of the burned patient: In the publication of Drago et al. (including this author), a new approach to the burned patient is proposed: After quick admission to the hospital, dead tissue should be removed as soon as possible. Thereafter, the skin barrier should be restored in a single procedure.[1,9,10] The novel proposed treatment includes the use of controlled enzymatic scarectomy in combination with “intelligent” polymeric films (Ifs) and “intelligent” scaffolds.[1,9,10] By using nanoparticles and/or fibers (NPFs), polymeric films or scaffolds can be loaded with bioactive agents and other drugs. These drugs or agents can selectively be released or delivered to the desired wound to stimulate wound healing.[1,9,10] In addition, the NPFs can stimulate selective anchoring and adhesion of endogenous circulating repairing cells, such as mesenchymal stem cells, to obtain a faster and more physiologic healing process.[1,9,10] Ultimately, improvements in tissue engineering and the use of nanoengineering will change the way acute burns are treated in the future and will eventually lead to effective and scar free healing.

Part 2. Discussion on keloid scars

Keloid scars are very difficult lesions to treat, since surgical excision alone results in a more than 60 percent recurrence rate.[11] Besides aesthetic deformity, these lesions can cause pain and pruritus and therefore an effective treatment is needed.[11] Current non-surgical treatments such as corticosteroid injections or silicone sheeting are not always effective and recurrence rates are high, especially in large or recalcitrant keloid scars.[11] This thesis evaluated current keloid scar treatment modalities, such as Intralesional cryotherapy and excision followed by adjuvant *radiation therapy (RT)*, which both proved to be effective in volume reduction and prevention of scar recurrence.[12,13] As a result, patients suffering from this benign lesions, can be treated more satisfactory.

A. Cryotherapy

Intralesional (IL) cryotherapy was designed to freeze the keloid scar from inside thereby destroying the core of the keloid, while at the surface, cells including melanocytes are much less affected.[14] As such, IL cryotherapy aimed to increase volume reduction, decrease recurrences while minimizing the risk of hypopigmentation as compared to traditional external cryotherapy.[15] In literature, promising results are published including no recurring scars and no persistent hypopigmentation following treatment.[15]

After an extensive evaluation in our treatment center, we found a similar volume decrease and alleviated complaints of pain and pruritus with the use of two different systems (liquid nitrogen-based and argon gas-based system).[13,16] However, in contrast to the published studies, recurrences and persistent hypopigmentation occurred up to 12 months following treatment with both systems.[13,16] We believe that these differences may be explained by the fact that our prospective studies used clear definitions for recurrence and scar inclusion, used a 12 months follow-up and included patients with only keloid scars consisting of all Fitzpatrick skin types (Fitzpatrick 1-6).

The argon device displayed a lower recurrence rate, but more hypopigmentation up to 12 months following treatment compared to the liquid nitrogen-based system. These different outcomes can be explained by differences in the thermal characteristics of both systems. Our hypothesis was that the argon gas system had a better freezing capacity with higher freezing rates and a colder tissue end temperature compared to the liquid nitrogen system. This leads to more necrosis in the core of the keloid, resulting in a low recurrence ratio. However, it may have also attributed to the higher hypopigmentation and wound dehiscence ratio seen following treatment.[17]

Finally, in contrast to other authors[18,19], we believe that some degree of melanocyte destruction is inevitable with the use of IL cryotherapy.[17] This was confirmed in our experimental study, in which surface temperatures below -20 °C were measured during treatment with both systems.[17]

B. Excision with adjuvant irradiation

Excision of a keloid scars is in most cases a good option to achieve an aesthetic good result: All the keloidal tissue is removed and the wound can be closed resulting in only a fine linear scar. However, since surgical excision alone results in >60% recurrence rate, adjuvant therapy following surgical excision is required.[11] This thesis extensively evaluated the use of excision with post-operative irradiation and proved it's highly effectiveness and low recurrence rate following treatment.[12,20]

As seen in this thesis, a very low recurrence rate in combination with a minimal complications ratio was obtained. We believe that this is due to a few reasons:

Firstly, we believe that the use of HDR brachytherapy compared to traditional external RT results in a more localized and precise irradiation of the residual scar. Moreover, a lower total radiation dosage is required. In this way, damage to healthy adjacent tissue is limited and the risk of complications is reduced. Factors that both can attribute to scar recurrence. We believe that minimalizing the total radiation dosage is very important, since our patients are in general healthy and suffer from a benign disease.

Secondly, our hypothesis is that through immediate post-operative radiation, immune cells are prevented from invading and proliferating into the lesion, thereby preventing scar recurrence. This hypothesis is also supported by several other groups treating keloids immediately after surgery or even perioperatively.[21-23]

C. Treatment algorithm for keloid scars

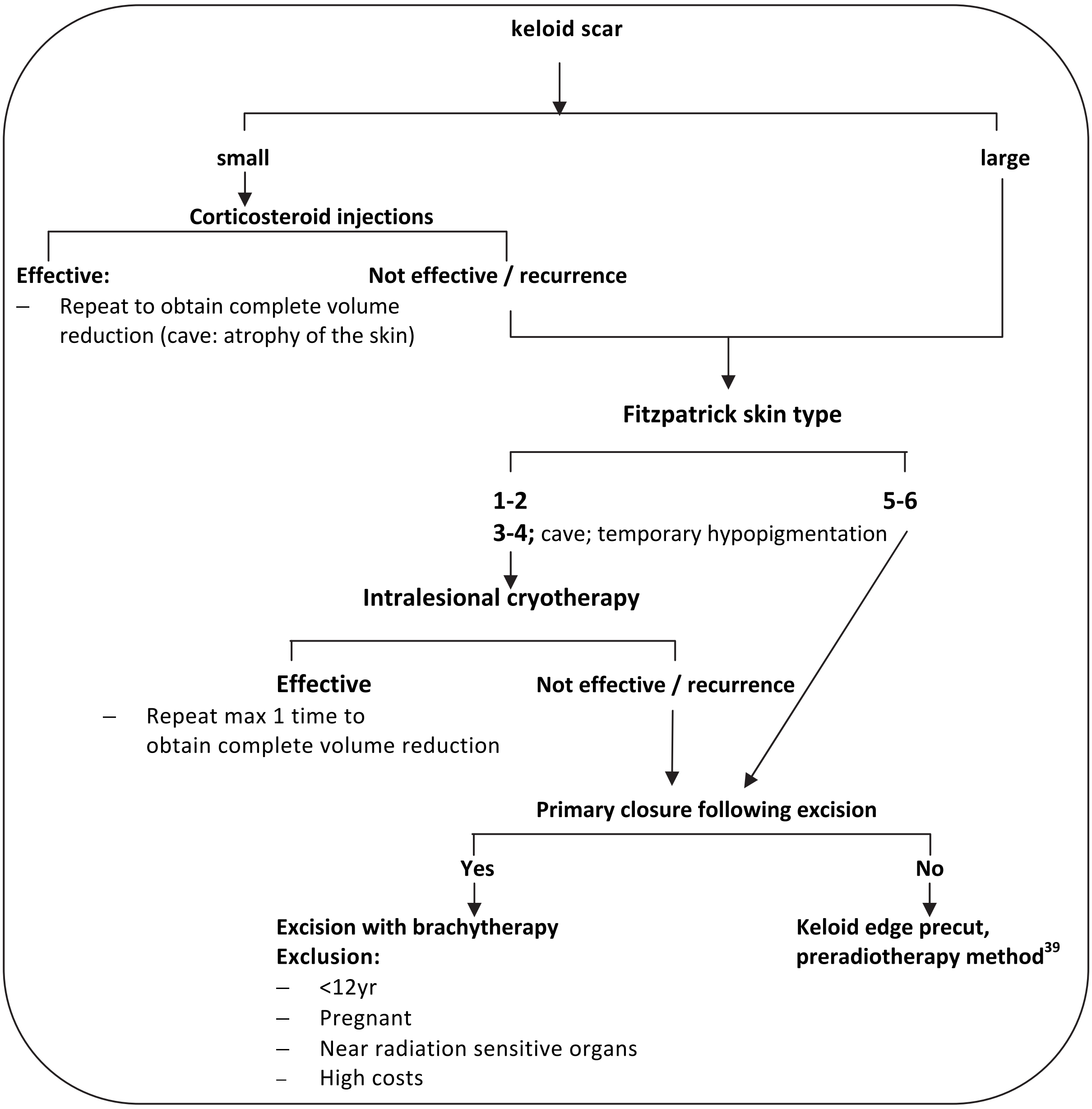
For the clinician in the field it is still difficult to choose the optimal treatment modality when treating a patient suffering from keloid scars.[24] As a result, they avoid treating keloid scars because of their high frequency of recurrence.[24] This thesis evaluated current keloid scar treatment modalities, such as Intralesional cryotherapy and excision followed by adjuvant RT, which both proved to be effective in volume reduction and prevention of scar recurrence.[12,13] Both therapies are the most effective if used under the following conditions:

- IL cryotherapy could be an addition to the existing keloid scar treatments: 1) If non-surgical techniques have failed. 2) As combination therapy with (non) surgical therapies as steroid injections or silicone gel sheeting. 3) As alternative for excision with adjuvant irradiation if there is no radio therapeutic capacity or the patient (<12yr) or keloid (size, anatomical location) is not suitable for RT. 4) In a specific subgroup of patients seeking for alleviation of pain and pruritus rather than complete scar eradication. 5) in Caucasian patients. Non-Caucasian patients should be avoided or warned for (temporary) hypopigmentation
- Surgical excision with irradiation is not always possible due to the patient characteristics (pregnant or age <12yr) or the location of the keloid scar (radiosensitive locations such as the thyroid gland). Also, the costs of excision with irradiation exceed the costs of other treatments significantly. Therefore, excision with adjuvant RT should be regarded as a

'last resort' for (recalcitrant) large keloid scars, when other non-surgical treatments have failed.

To provide an overview of possible keloid scars treatments, we present a keloid scar treatment algorithm (figure 1) based on the algorithm as proposed by Ogawa.[24]

Figure 1. Treatment algorithm for keloid scars.



Future directions on keloid scars

With concerns to IL cryotherapy, novel systems or adjustments of the existing systems are required to obtain complete scar eradication, lower the recurrence rates and to control hypopigmentation. Also, randomized studies are needed to generate stronger evidence proving the effectiveness of IL cryotherapy. Such studies should include non-Caucasian patients, as they are more prone to pigmentation problems[26] and at higher risk of persistent hypopigmentation.[13]

Regarding excision and adjuvant irradiation, this thesis presents one of the lowest total dose radiation protocol described in literature. Our radiation protocol required patients to return only once to the outpatient clinic for second radiation treatment, thereby enhancing patient convenience. Future research should focus on optimizing the radiation protocol. For example, a single radiation scheme could be used or perioperative or even preoperative radiation could be considered. Also, the total dose could potentially be further reduced. In this way, radiation damage to healthy surrounding tissue is reduced and potential radiation related complications are minimalized.

Because of the heterogeneity in study design and outcome measures between studies evaluating keloid scar treatments, it is still difficult to determine effectiveness of the various keloid scar treatments.[11,24] To make comparison between studies and different treatment modalities possible, future studies should use reliable and validated objective and subjective measurement devices to determine the outcomes following treatment. In addition, the following aspects regarding study design should be respected: 1) Studies should be prospective of nature and preferably randomized. 2) Follow-up should have a minimum of 12 months, but preferably 2 or even 3 years to determine the recurrence ratio. 3) A definition of scar recurrence should be included. 4) A definition of keloid scars should be included in the study protocol. This is relevant, since hypertrophic scars have better prognostic factors regarding recurrence rate compared to keloid scars. Also, post-treatment histology of the excised lesion may be used to determine the nature of the scar. 6) Inclusion of a variety of Fitzpatrick skin type (F1-6)[26] patients is essential since Afro-American patients (F5-6) are more prone to pigmentation disorders[25,27] and scar recurrence[28] compared to Caucasian patients (F1-3).

Finally, this thesis presented current keloid scar treatment modalities, which both proved to be effective in volume reduction and prevention of scar recurrence.[12,13] As a result, patients suffering from this benign lesions, can be treated satisfactory. The main question however in current and future keloid scar research will be to reveal the working mechanism behind these techniques and, in general, the working mechanism behind keloid formation and its recurrence after resection.[29,30] Also, preferred localization of keloid formation and increased incidence among different patient groups, as seen in this thesis[13,16], are still not clarified.[29,30] The following hypotheses may be used as basis for future research revealing the working mechanism behind scar formation and scar recurrence:

As described earlier, cryoablation directly induces necrosis by damaging cell membranes and organelles via the formation of ice crystals, and indirectly through osmotic stress and ischemia from thrombosis of the microvasculature.[31–33] In this manner, the keloidal tissue is destroyed leading to a (total) volume reduction and deterioration of complaints of pain and pruritus.[13,16] It is however unclear in which way cryotherapy prevents the scar from recurring. After all, IL cryotherapy causes trauma to the skin by introducing the needle and causes damage through cryodestruction of the tissue. Normally, keloid scars develop after such trauma of the skin; in clinical practice however, few recurrence.[34]

Although authors described cryotherapy to induce a new scar without keloidal characteristics, no closing evidence has been presented.[35] This thesis adds therefore a hypothesis: After burn trauma, all cells and connective tissue matrix are completely destroyed.[36,37] During the healing process, the wound is filled with granulation tissue and the wound is finally closed through contraction. After healing, wound contractions and scar hypertrophy causes major physical problems. Remarkably, after freezing injuries, no wound contraction is reported and the connective tissue matrix is maintained.[36,37] This lack of contraction may be the explanation for the prevention of scar recurrence following cryotherapy. The hypothesis is that the devitalized residual scar, as formed following cryotherapy, will act as a scaffold. This scaffold allows the influx of new cells and more importantly, prevents the wound from contraction. This lack of wound contraction may prevent the formation of keloidal cells, since these cells are prone to tension.[11]

Another potential working mechanism of the prevention of keloid recurrence following cryoablation contains the activation of a cryo-immunologic response;[31,32] In the past decade, the use of cryoablation for cancer therapy has expanded.[31,32] Cryoablation is used for the ablation of prostate, liver, breast and lung tumors. Besides the already described direct necrotic effect of cryoablation, another potential benefit has been revealed in studies investigating cancer therapy. Since the tumor is not surgically resected, but is left in situ for the body to absorb after cryoablation, it is hypothesized that the frozen tissue has an ability to stimulate an immunologic response to tumor-specific antigens. Reports have been published in which metastatic foci regressed after cryoablation of a primary tumor, suggesting a potential systemic benefit to a local therapy.[32] In current keloid scar research, the formation of keloidal tissue is frequently linked to an immunologic disorder. Cryoablation may therefore not only debulk the keloidal tissue, but the necrotic keloidal tissue may also become a rich reservoir of keloid-associated antigens thereby boosting an immune response which may prevent keloid scar recurrence following cryoablation. With concerns to surgical excision and adjuvant RT results in a complete resection of the scar, with a recurrence rate of 4%.[12] It is likely that the adjuvant RT prevents the scar from recurrence since surgical excision alone results in a more than 60% recurrence rate.[11]

RT is considered the primary non-surgical modality in the curative treatment of cancer.[38] Recent data suggested that RT can modulate anti-tumor immune responses, modifying the tumor and its microenvironment. Just as with cryoablation, RT may boost an immune response which may prevent keloid scar recurrence following surgical resection.

Revealing the magic behind these keloid scar treatment modalities may have a huge impact of our current understanding of the etiology of keloid scars. It could contribute to the ultimate goal of effective keloid scar treatment without recurrence and even towards prevention of these lesions.

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Summary

The skin is one of the most important organs of our body, but it is also vulnerable and can easily get damaged. After healing of the damaged skin, scarring is likely to be present. In some cases and in some individuals, abnormal or excessive scarring following damage occurs.

Abnormal scarring can cause major physical complaints and aesthetic disfigurement, hence impairing the quality of life of the patient. This thesis describes current scar modalities for the treatment of such abnormal scars. The focus lies on the prevention of hypertrophic scars following acute burn wounds or reconstructive wounds (Part I). In addition, novel keloid scar treatments are evaluated (Part II + III)

Part I. Dermal substitutes for treatment of acute burns and reconstructive wounds

Due to improvements in medical health care, the mortality rate following large burn trauma decreased. However, severe scarring is still seen and therefore new challenges within current burn trauma medicine have risen. Until now, grafting of the burned area with autologous split thickness skin grafts (SSG) is the gold standard. However, severe scarring following grafting with SSG's is seen. The severe scarring is thought to be due to the lack of dermal tissue in the grafts, which initiated the development of dermal substitutes.

In **chapter 2**, we outline the biological background of the three main classes of dermal substitutes. Furthermore we relate several characteristics to clinical requirements and discuss the current available dermal substitutes.

In **Chapter 3**, the clinical implementation of such a dermal substitute is evaluated in a long-term follow-up. This study represents the first long-term and objective follow-up of a dermal substitute in which its effectiveness was investigated in acute and reconstructive burn surgery. Seventy-nine percent of the patients of the original study of Van Zuijlen et al, were screened twelve years post-treatment. Both subjective and objective evaluation tools were used. The study shows that even after twelve years, a higher elasticity was seen in reconstructive wounds treated with the dermal substitute compared to the non-substituted reconstructive wounds.

Part II Intralesional cryotherapy for treatment of keloid scar

Intralesional (IL) cryotherapy is a promising treatment technique in which the scar tissue is frozen from *within* the lesion. We evaluate IL cryotherapy in a comprehensive review including all clinical studies (**chapter 4**). Clinically, two different systems were tested in a prospective study; in **chapter 5**, we evaluate a liquid nitrogen-based system and in **chapter 6** we tested an argon gas-based system, which had never been used before for treatment of keloid scars. Compared to the liquid nitrogen system, the argon gas system provided a lower recurrence percentage, but more hypopigmentation post-treatment.

To explain the different outcomes between the liquid nitrogen and the argon gas system, more information about the exact working mechanism of IL cryotherapy was required. Therefore we designed an experimental study, investigating the argon gas and liquid nitrogen system ex vivo and in vivo (**chapter 7**). In this study the argon gas system showed to reach lower end temperatures and a faster freezing rate compared to the liquid nitrogen system.

Part III Excision with adjuvant irradiation for treatment of keloid scars

Treatment of keloid scars is difficult with high recurrence rates and even growth stimulus as the main issue. According to the international advisory panel on scar management, surgical excision with post-operative radiation therapy is considered the most efficacious treatment.

In **chapter 8**, we present a systematic review according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA). This review proved brachytherapy to show lower recurrence rates compared to external radiation. Also, a short time (<7hrs) interval between scar excision and irradiation constituted a lower recurrence rate compared to longer time-intervals (>24hrs). Single-fraction irradiation showed promising results in terms of recurrence rate and patient convenience. Finally, scar recurrence was seen between 2 and 36 months, with a mean of 15 months.

In **chapter 9**, we present our ten years' experience with a brachytherapy out-patient procedure. The treatment protocol employed a unique radiation schedule with a total dose of 2x6=12 Gray. Importantly, the first dose was administered immediately post-operative and is the lowest dosage known in literature for the treatment of keloids. In a long-term prospective study, we evaluated the effectiveness of this radiation schedule. After 33.6 months we found a very low recurrence percentage of 3.1%. Also, complaints of pain and pruritus decreased with 82.9% and 87.2% respectively. The unique radiation schedule proved the efficacy and safety of HDR brachytherapy and suggested the importance of immediate postoperative.

Chapter 10 presents a novel method to treat (very) large keloids, which cannot be treated with excision followed with brachytherapy

Finally, in **chapter 11,** the most important findings of this thesis and our clinical experience with the devices and are discussed. Lastly, future perspectives are presented in which we direct future research towards the ultimate goal of scar free healing and prevention of even towards prevention of aberrant scar formation.

Samenvatting

De huid is het grootste en één van de belangrijkste organen van het menselijk lichaam. Echter, het is ook een van de meest kwetsbare organen en kan eenvoudig beschadigd raken.

Als de huid beschadigd wordt, volgt er een genezingsproces wat in de meeste gevallen leidt tot littekenvorming. In sommige gevallen (brandwondenslachtoffers), bij bepaalde personen (erfelijk belast) of bij bepaalde bevolkingsgroepen (negroïde bevolking), kan er echter sprake zijn van overgenezing. Hierbij is de wondgenezing verstoord geraakt, resulterend in een excessieve toename van littekenweefsel. Deze excessieve toename van littekenweefsel kan resulteren in twee soorten littekens: hypertrofische littekens en keloïd littekens. In het geval dat een dergelijk litteken ontstaat, kan dit leiden tot functionele, cosmetische en psychologische problemen.

In dit proefschrift ligt de focus enerzijds op de preventie van hypertrofische littekens bij brandwonden en reconstructieve wonden (Deel I). Anderzijds worden er diverse behandeling methoden voor keloïd littekens geëvalueerd (Deel II+III).

Deel I Dermale substitutie voor de behandeling van brandwonden en reconstructieve wonden

Verbeteringen in de behandeling van acute brandwonden hebben geleid tot een aanmerkelijk hoger overlevingspercentage. De standaardbehandeling van diepe brandwonden bestaat uit een huidtransplantatie. Deze huid wordt van een onaangedaan gedeelte van de patient afgenomen en op de brandwond geplaatst. Alhoewel met deze behandeling een hogere overlevingskans is bewerkstelligd, wordt er nog steeds zeer ernstige littekenvorming gezien na genezing. Deze ontwikkeling heeft gezorgd voor nieuwe uitdagingen in de brandwondenzorg. Inmiddels is namelijk bekend dat het herstel van de dermis belangrijk is voor de kwaliteit van het litteken. Daarom is er de laatste jaren een grote ontwikkeling geweest in het maken van kunstdermis, oftewel een dermaal substituut. Het gebruik van een dermaal substituut zou zorgen voor een verbeterde functionele en cosmetische littekenkwaliteit.

In **hoofdstuk 2** wordt het gebruik van dermale substituten uitgebreid geëvalueerd. De biologische achtergronden en principes van de drie groepen dermale substituten worden besproken. Daarnaast zijn de basisprincipes beschreven waaraan een dermaal substituut moet voldoen om klinisch hanteerbaar te zijn. Tenslotte is de huidige literatuur doorgenomen en worden alle momenteel beschikbare dermale substituten weergegeven.

In **hoofdstuk 3** is de klinische implementatie van een dermaal substituut genaamd Matriderm geëvalueerd. Deze studie beschrijft een 12-jaars controle na een behandeling met een dermaal substituut bij patiënten met acute brandwonden en reconstructieve wonden. Zevenennegentig procent van de patiënten werd teruggezien 12 jaar na behandeling en de wonden werden gemeten middels subjectieve vragenlijsten en objectieve meetapparaten. Zelfs na 12 jaar werd er nog steeds een hogere mate van elasticiteit gezien bij de wonden die een dermaal substituut hadden gekregen, vergeleken met de controle wonden.

Deel II Intralesionale cryotherapie voor de behandeling van keloïd littekens

Intralesionale (IL) cryotherapie is een veelbelovende nieuwe techniek voor de behandeling van keloïd littekens. Hierbij wordt het keloïd door een holle naald van binnenuit bevroren met vloeibaar stikstof. In **hoofdstuk 4** wordt IL cryotherapie geëvalueerd in een overzichtsartikel wat de huidige literatuur in kaart brengt en bespreekt.

In **hoofdstuk 5 en 6** worden de resultaten van 2 verschillende bevriezingsapparaten (gebaseerd op vloeibaar stikstof én gebaseerd op argon gas) besproken.

In **hoofdstuk 7** worden beide apparaten vergeleken in een experimentele studie waarin de thermale karaktereigenschappen van beide apparaten bepaald zijn.

Deel III Excisie met aanvullende bestraling voor de behandeling van keloïd littekens

Indien non-chirurgische behandelingsmethoden resulteren in een slecht resultaat of een recidief van het keloïd litteken, is chirurgische excisie een laatste optie. Echter is een aanvullende behandeling na het excideren vereist. Dit omdat excisie alleen leidt tot een recidiefpercentage van meer dan 60%. Gebleken is dat de meest effectieve aanvullende behandeling bestaat uit het bestralen van de huid.

In **hoofdstuk 8** wordt een overzicht gegeven van de verschillende huidige bestralingsmethoden. Uit dit overzicht bleek dat bestraling met brachytherapie (inwendige bestraling) een lager recidief percentage oplevert dan traditionele uitwendige bestraling. Ook bleek dat een vroege postoperatieve bestraling eveneens zorgt voor een laag

recidiefpercentage. Tenslotte adviseren wij om patiënten minimaal 15 maanden na de behandeling terug te zien, om de meeste recidieven te kunnen objectiveren.
In **hoofdstuk 9** worden de resultaten gepresenteerd van een studie waarin patiënten werden behandeld met excisie van het keloïd litteken gevolgd door brachytherapie. Een noviteit was dat patiënten meteen na de operatie de bestraling toegediend kregen en maar eenmaal terug hoefden te komen voor een laatste aanvullende bestraling. Niet alleen is dit patiëntvriendelijk, onze hypothese is ook dat directe bestraling de kans op een recidief vermindert.

In **hoofdstuk 10** wordt een methode beschreven om ook grote keloïden te kunnen bestralen, die niet in één keer gesloten kunnen worden na excisie.

Tenslotte worden in **hoofdstuk 11** de meest belangrijke uitkomsten van dit proefschrift en onze eigen klinische ervaringen met de materialen en apparaten besproken. Afsluitend worden aanbevelingen gedaan voor toekomstig onderzoek, daarbij strevend naar littekenvrije wondgenezing en preventie van overmatige littekenvorming.

Dankwoord

De totstandkoming van mijn promotie was niet mogelijk geweest zonder de bijdrage en steun van een aantal mensen en instanties.

In de eerste plaats zijn dit de patiënten die herhaaldelijk tijd hebben vrijgemaakt om vragenlijsten te beantwoorden, hun littekens te laten opmeten en het vertrouwen hebben gehad in nieuwe behandelingsmethoden. Graag wil ik u allen hiervoor danken.

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The **Review Committee**. Prof. dr. C.M.A.M. van der Horst, Prof. dr. B. van der Lei, Prof. dr. Y. Har-Shai, Prof. dr. R.R.W.J. van der Hulst, Prof. dr. H.J. Bonjer and Prof. dr. S. Monstrey. I am honored that you were willing to be member of the Review Committee. Thank you for reading and reviewing my thesis and for travelling to Amsterdam for the defense.

Brandwondencentrum Rode Kruis Ziekenhuis, Prof. dr. P.P.M. van Zuijlen en Prof. dr. E. Middelkoop. Beste Paul en Esther, samen met mijn huidige paranimf **Niels van Vucht** begonnen bij jullie op de afdeling in het team met Martijn, Monica en Paulien. Onder het motto ‘meten is weten’ heb ik hier ontzettend veel geleerd over het opzetten en uitvoeren van wetenschappelijk onderzoek op het gebied van littekens. Hartelijk dank hiervoor!

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Lieve Steffie, jij bent de allerbeste steun die ik mij kan voorstellen. Samen hebben we verdriet, teleurstellingen en successen doorgemaakt. Samen hebben we, vaak met verbazing, de medische wereld aanschouwd, doorgenomen en beoordeeld.
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Ik hou ontzettend veel van jou en ben dol op je!

Curriculum Vitae

Michiel Christiaan Evert van Leeuwen was born on the 4th of March 1987 in Amsterdam. At the age of six, Michiel moved to Bloemendaal with his family. He graduated his gymnasium degree at the Stedelijk Gymnasium Haarlem in 2005.

In the same year, he enrolled medical school at the VU University in Amsterdam. During his study, Michiel was active within his student organization by organizing the anniversary year and by co-founding the 'Geneeskundig Symposium Amsterdam'. Also, he started several small businesses such as selling and organizing ski tours for student groups and a transport company named LIJN20.

During his second year of medicine, Michiel was inspired by a lecture about the treatment of burn scars. He decided to contact the professor to see whether he could join the research team. In the following years, Michiel performed research at the Burn Scar Centre in Beverwijk, the Netherlands and at the Hospital de Quemados (burns hospital) in Buenos Aires, Argentina. During his clinical rotations, Michiel continued his research activities at the Plastic Surgery department at the VU Medical Center, where he evaluated several keloid scar treatments.

After his graduation from medical school, Michiel worked for one year as a resident (ANIOS) at the trauma department of the HagaZiekenhuis (Haga Teaching Hospital) and the Juliana Children's Hospital in The Hague.

In July 2013, he decided to pursue his ambition to finish his research activities. Now, six years after his initial student research project in 2009, Michiel will be defending his thesis 'Prevention and Treatment of Scars' in July 2015.
To fund his PhD candidacy, Michiel worked part-time as a resident (ANIOS) in the addiction treatment center of Novadic-Kentron and started his own clinic in cosmetic medicine (ML Clinics).

Michiel decided to expand his horizon and will start as an International Medical Advisor at Novo-Nordisk at the global head office in Copenhagen, Denmark.

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